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ANCHORAGE AND EXTENT OF CORN ROOT SYSTEMS[1]

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INTRODUCTION

Results from preliminary experiments indicate that there are fundamental differences in the root systems of various inbred strains of corn. The object of the present paper is (1) to call attention to a plant-pulling machine which was designed for these studies and (2) to point out a few significant differences in the root systems of the inbred strains that were included in the experiments.

MATERIALS AND METHODS

A plant-pulling machine (Pl. 1) was designed to measure the resistance of individual plants or hills of corn to a vertical pull. The essential features of this machine are a cross beam with three axes which divide the beam into two parts, so as to make a lever of the second class. Each axis consists of a knife edge turning in a clevis. The distal axis is attached to a spring balance which registers the pulling resistance to the tenth of a pound. The clevis of the other end of the beam is supported by a block and tackle, the rope being pulled by winding it on a 2-inch drum. Two thicknesses of strong cotton webbing such as are used for trunk straps were attached to the third clevis, and these were attached to the base of the corn stalks by a suitable hitch.

The inbred strains of corn, except where indicated otherwise in the tables, had been selfed five and six years. The strain, designated as "good," not only possessed the ability to stand erect under adverse weather conditions, but was exceptionally good in general vigor, chlorophyll development, and resistance to fungus diseases, including rootrot and smut. The strain susceptible to rootrot was equally good in general vigor, and the leaves were free from leaf spotting and other chlorophyll deficiencies, but plants of this strain lodged badly during windstorms. At no time did the leaves of either of the above strains show any tendency to wilt during early or mid-season periods. Leaves of the strain susceptible to leaf firing invariably wilted during any dry, hot period in July. Severe wilting of the leaves was followed by a dying of portions of those leaves.

The field data were secured from plats having a perfect stand of one plant every 22 inches in rows 42 inches apart. The plants grown in the greenhouse were spaced 18 inches each way.

The significance of differences in means was determined by "Student's" method,[3] wherever that method could be applied. Differences with odds of 30:1, were considered significant.

[1] Accepted for publication Nov. 19, 1923.

[2] The investigations reported in this paper were conducted in cooperation with the Illinois Agricultural Experiment Station and Funk Bros. Seed Co., of Bloomington, Ill.

[3] THE PROBABLE ERROR OF A MEAN. *In* Biometrika, v. 6, p. 1–25. 1908. (Signed by Student.)

EXPERIMENTAL DATA

ROOT ANCHORAGE STUDIES

The results presented in Table I were secured under very uniform conditions. Previous to planting the corn, the large soil pit in one of the greenhouses at the University of Illinois was refilled with new soil which had been thoroughly mixed. Although plants of the strain susceptible to rootrot had not been exposed to wind and storm and had equal opportunities to develop a strong root system, yet the mean pull of these plants was less than half that of plants of the good strain, 13.8 ± 1.1 pounds compared with 29.8 ± 1.8 pounds, respectively. Plants of the strain susceptible to leaf firing offered less than one-third the resistance to a vertical pull that was exhibited by plants of the good strain, 9.1 ± 0.9 pounds compared with 29.8 ± 1.8 pounds, respectively. There was no material difference in the height of plants of these three strains on the date when the plants were pulled. None of the roots showed any evidence of root rotting. Leaves of the strain susceptible to leaf firing apparently were normal in every respect. In view of such facts, the differences of 16.0 ± 2.1 pounds and 20.7 ± 2.0 pounds in root anchorage are very significant. The two first generation hybrids were somewhat more vigorous than the good inbred strain as measured by vegetative growth, but they were not superior in pulling resistance. These data suggest that the genetic factors responsible for the reduced root systems in the strains susceptible to rootrot and leaf firing, respectively, are recessive. Repeated field experiments not herein reported confirm this suggestion.

The plants of a number of inbred strains were pulled after they had matured in field plats, but while the stalks were still green. The results given in Table II show that erect plants of the good strain were better anchored than erect plants of the strain susceptible to rootrot. The difference of 48.6 pounds, or 23.4 per cent, with odds of 132:1, is sufficiently large to be significant.

TABLE I.—*Data on the root anchorage of three unrelated selfed strains of Yellow Dent and three F_1 crosses measured by their resistance to a vertical pull, the corn being planted Mar. 8, 1922, in the greenhouses of the University of Illinois and pulled May 6, 1922*

Character of inbred strains and crosses.	Number of plants pulled.	Mean pulling resistance per plant.	Difference in pulling resistance based on good strain.		Difference / P. E.
		Pounds.	*Pounds.*	*Per cent.*	
Good	23	29.8 ± 1.8	.....	.....	.....
Susceptible to rootrot	22	13.8 ± 1.1	16.0 ± 2.1	53.7	7.6
Susceptible to leaf firing	25	9.1 ± 0.9	20.7 ± 2.0	69.5	10.3
F_1 (Good x rootrot susceptible)	26	29.4 ± 1.6	$.4 \pm 2.4$	1.3	.2
F_1 (Good x leaf firing susceptible)	26	28.3 ± 1.6	1.5 ± 2.4	5.0	.7

TABLE II.—*Data on root anchorage of twenty-eight erect plants of a good inbred strain and a similar number of erect plants in an adjacent row of an inbred strain susceptible to rootrot, the corn being planted May 30, 1922, near Bloomington, Ill., in brown silt loam soil, and root anchorage data taken September 26, 1922*

Character of strain.	Number of plants pulled.	Mean pulling resistance per plant.	Difference in pulling resistance.		Odds.
		Pounds.	*Pounds.*	*Per cent.*	
Good	28	208.0	.....	.....	.....
Susceptible to rootrot	28	159.4	48.6	23.4	132:1

The relation of root anchorage to lodging is shown by data presented in Table III. As the mean pulling resistance of erect plants decreased,

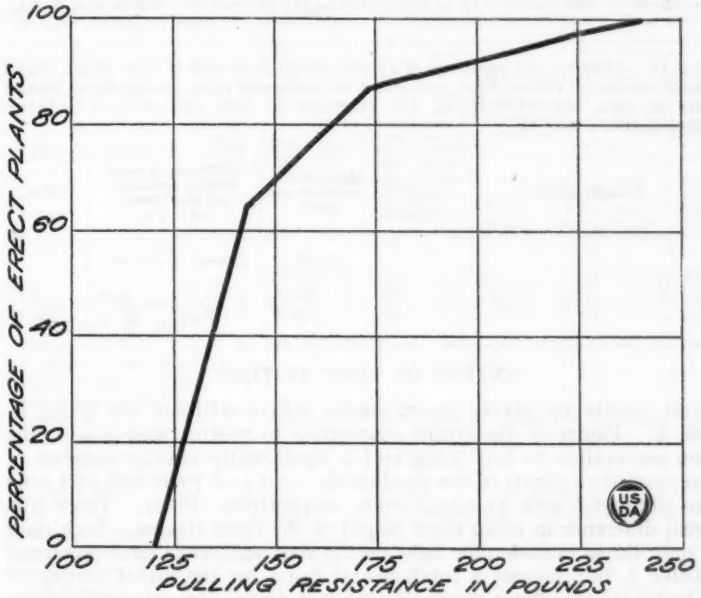


FIG. 1.—Graphic representation of data in Table III, showing increase in force necessary to uproot corn plants as the percentage of erect plants increased.

the percentage of leaning plants increased. These data are presented graphically in figure 1.

Strains which have the ability to stand erect throughout the season may vary greatly in their resistance to a vertical pull, as shown by data presented in Table IV. The difference of 32.7 pounds, or 9 per cent, in pulling resistance between G–4–2 and G–4–3 is not significant in view of the small odds involved. The difference of 118.9 pounds, or 32.9 per cent, however, between G–4–2 and G–4–4, with odds of 999:1, is very significant.

TABLE III.—*Data on relation of anchorage to lodging in some closely related inbred strains of Yellow Dent corn, as measured by their resistance to a vertical pull, the corn being planted May 22, 1922, near Bloomington, Ill., in brown silt loam soil, and plants pulled September 27–28*

Pedigree number.	Number of plants pulled.	Mean plant height.	Plants leaning 30° or more.		Mean pulling resistance per plant.		Difference in mean pulling resistance per plant based on B-1-1-1-R-3.			
					Erect plants.	Mean of both erect and leaning plants.	Erect plants.	Difference/ P. E.	Both erect and leaning plants.	Difference/ P. E.
		Inches.	*Number.*	*Per cent.*	*Pounds.*	*Pounds.*	*Pounds.*		*Pounds.*	
B-1-1-1-R-3..	37	82.6±1.1	0	0.0	240.0±7.1	240.0±7.1	.....	.....	.....	.....
B-1-1-1-R-7..	40	96.0±0.8	1	2.5	228.0±6.1	224.6±6.0	12.0± 9.4	1.3	15.4± 9.3	1.6
B-1-1-1-R-8..	36	95.4±1.3	5	13.9	183.9±9.4	173.0±9.0	56.1±11.8	4.7	67.0±11.5	5.8
B-1-1-1-R-10.	39	87.3±0.7	14	35.9	155.3±10.0	144.3±7.5	84.7±12.2	6.9	95.7±10.3	9.3
B-1-1-1-1-2...	25	89.6±1.0	25	100.0	.....	120.1±8.1	.....	.....	119.9±10.8	11.1

TABLE IV.—*Data on root anchorage of 25 erect plants from each of three closely related inbred strains of Yellow Dent corn grown in contiguous rows, the corn being planted May 22, 1922, near Bloomington, Ill., in brown silt loam soil, and root anchorage data taken September 28*

Pedigree number.	Number of plants.	Mean pulling resistance per plant.	Difference in mean pulling resistance per plant based on G-4-2.		Odds.
		Pounds.	*Pounds.*	*Per cent.*	
G-4-2	25	361.5	.....	.....	.....
G-4-3	25	328.8	32.7	9.0	5 : 1
G-4-4	25	242.6	118.9	32.9	999 : 1

EXTENT OF ROOT SYSTEMS

Root counts on plants grown under field conditions are given in Table V. Plants of the strain susceptible to rootrot and also of the strain susceptible to leaf firing had a significantly smaller number of main roots than plants of the good strain, 20.0±1.6 roots and 15.4±0.7 roots compared with 33.0±0.8 roots, respectively (Pl. 2). There was no real difference in mean plant height of the three strains. Such data not only throw considerable light on the root-anchorage data presented in Table I, but suggest a fundamental cause for the initial wilting of the leaves of the strain susceptible to leaf firing. In this strain there seemed to be an actual deficiency in the root system compared to the vegetative growth above ground. During periods of rather low soil moisture and high transpiration, such a small root system could hardly be expected to supply the needs of the plant. Differences in the root systems of the good strain and the strain susceptible to leaf firing are illustrated in Plate 2.

The results from root examinations made August 17–25 are given in Table VI. There was a great increase in growth in all parts of the plant during the period between July 18 and August 17–25. The great increase in number of roots is in accord with the findings of Weaver, Jean, and Crist.[4]

[4] WEAVER, John E., JEAN, Frank C., and CRIST, John W. DEVELOPMENT AND ACTIVITIES OF ROOTS OF CROP PLANTS. vi, 117 p., 42 fig., 14 pl. Washington, D. C. 1922. Bibliography, p. 116–117. (Carnegie Inst. Wash. Pub. 316.)

TABLE V.—*Data from a comparative study of the root systems of three unrelated inbred strains of Yellow Dent corn planted June 2, 1922, at Bloomington, Ill., in brown silt loam soil that previously had produced two crops of corn, and excavated July 18, 1922*

Character of inbred strains.	Number of plants examined.	Mean plant height.	Mean number main roots per plant.	Difference in number of main roots per plant based on good strain.		Difference/P.E.
		Inches.		*Number.*	*Per cent.*	
Good	4	59.0±1.1	33.0±0.8			
Susceptible to rootrot	7	59.3±1.0	20.0±1.6	13.0±1.8	39.4	7.2
Susceptible to leaf firing	9	57.2±2.4	15.4±0.7	17.6±1.1	53.3	16.0

TABLE VI.—*Data from a comparative study of three unrelated inbred strains of Yellow Dent corn planted June 2, 1922, at Bloomington, Ill., in brown silt loam soil that previously had produced two crops of corn, and excavated August 17–25*

Character of strain.	Number of plants examined.	Mean height.	Mean circumference.	Mean number of main roots.	Mean percentage of rotted roots 10 inches below surface.	Mean number of lateral roots per plant on six 6-inch pieces of main roots.		Mean total length of all lateral roots per plant on six 6-inch pieces of main roots.	
						2–8 inches below surface.	10–16 inches below surface.	2–8 inches below surface.	10–16 inches below surface.
		Inches.	*Inches.*					*Inches.*	*Inches.*
Good	9	99.0	3.41	58.5	9.7	391.0	244.6	774.7	423.6
Susceptible to rootrot	9	109.1	3.45	51.7	96.7	293.1	96.0	334.4	103.5
Susceptible to leaf firing	9	102.0	3.40	57.0	4.0	391.0	160.2	423.7	208.9
Difference between good strain and strain susceptible to rootrot		10.1		6.8	87.0	97.9	168.6	330.3	320.1
Percentage difference between good strain and strain susceptible to rootrot		10.2		11.6		25.0	69.0	43.1	75.6
Odds		9,999:1		59:1	525:1	1,492:1	348:1	1,999:1	4,544:1
Difference between good strain and strain susceptible to leaf firing							84.4	351.0	214.7
Percentage difference between good strain and strain susceptible to leaf firing							34.5	45.3	50.6
Odds							344:1	81:1	1,350:1

The difference of 6.8 in mean number of main roots between the good strain and the strain susceptible to rootrot, although not large, is rather significant in view of the odds of 59 to 1. Plants of the strain susceptible to leaf firing showed a very large gain in number of main roots during the period between July 18 and August 25. The number of rotted roots in the strain susceptible to rootrot was very pronounced. There was little evidence of rootrot in the strain susceptible to leaf firing.

Actual differences in root systems of the three strains could not be expressed fully by the number of main roots; there were significant differences both in number and length of lateral roots (Plate 3). There was a very significant reduction in number of lateral roots in the strain susceptible to rootrot, both 2 to 8 inches below the surface and 10 to 16 inches below the surface. The figures were 293.1 and 96 lateral roots compared with 391 and 244.6 roots, respectively. (Table VI.) Plants of the strain susceptible to leaf firing developed as many lateral roots near the surface as plants of the good strain, but there was a marked reduction in the number of lateral roots 10 to 16 inches below surface, or 160.2 lateral roots compared with 244.6 roots. Differences in total length of the lateral roots on the same number of main roots of an equal length were even more marked than differences in the number of lateral roots, and perhaps were more important from the standpoint of the physiology of the plants concerned. In the strain susceptible to rootrot there was less than half the total length of lateral roots 2 to 8 inches below surface and only one-fourth the total length 10 to 16 inches below the surface, or 334.4 inches and 103.5 inches compared with 774.7 inches and 423.6 inches, respectively. (Table VI.) Roots of the strain susceptible to leaf firing were decidedly inferior to roots of the good strain in this respect, or 423.7 inches and 208.9 inches compared with 774.7 inches and 423.6 inches, respectively. All the differences in mean total length of all laterals on equal lengths of main roots from the different strains were significant from the standpoint of statistical analysis.

TABLE VII.—*Data from a comparative study of 10 plants of a good inbred strain of Yellow Dent corn and 10 and 5 plants, respectively, from two unrelated inbred strains susceptible to rootrot, planted May 22, near Bloomington, Ill., in brown silt loam soil that previously had produced three consecutive crops of corn, and excavated September 11–14, 1922*

Character of strain.	Number of plants.	Mean plant height.	Mean plant circumference at base.	Mean total number of main roots per plant.	Mean total number of lateral roots per plant on six 6-inch pieces of main roots 6-12 inches below surface.	Mean total length of all lateral roots per plant on six 6-inch pieces of main roots 6-12 inches below surface.	Mean total length of all laterals per plant on healthy roots only, in a horizontal 6-inch section 6-12 inches below surface.
		Inches.	*Inches.*			*Inches.*	*Inches.*
Good	10	82. 8±1. 2	3. 78±0. 13	41. 2±0. 4	434. 5±33. 5	706. 1±91. 9	4, 343. 9±415. 1
(*a*) Susceptible to rootrot	10	102. 3±1. 2	3. 69±0. 11	35. 1±2. 0	297. 6±16. 1	258. 8±21. 8	1, 220. 2±130. 7
(*b*) Susceptible to rootrot	5	90. 8±3. 7	3. 70±0. 12	31. 0±1. 1	209. 8±23. 0	208. 6±35. 1	946. 8±153. 8
Difference between good and (*a*) susceptible strain	.....	19. 5±1. 7	0. 09±0. 2	6. 1±2. 0	136. 9±37. 2	447. 3±94. 3	3, 123. 7±435. 2
Percentage difference between good strain and (*a*) susceptible strain	.....	23. 5	.....	14. 8	31. 5	63. 3	72. 2
Difference/P. E.	.....	11. 5	.....	3. 0	3. 7	4. 7	7. 2
Difference between good and (*b*) susceptible strain	.....	8. 0±3. 9	0. 08±0. 2	10. 2±1. 2	224. 7±40. 6	497. 5±98. 4	3, 397. 1±482. 5
Percentage difference between good strain and (*b*) susceptible strain	.....	9. 7	.....	24. 8	51. 7	70. 4	78. 2
Difference/P. E.	.....	2. 1	.....	8. 5	5. 5	5. 1	7. 0

Additional data from a comparative study of root systems of plants from a good strain and two strains susceptible to rootrot are given in Table VII. Differences in the number of main roots, 6.1 ± 2.0 roots and 10.2 ± 1.2 roots, were marked. The latter difference, being 8.5 times its probable error, is very significant. Both differences in mean total number and mean total length of all laterals on equal lengths of main roots are very significant. The greatest and most important difference between the good strain and the strains susceptible to rootrot, however, lay in the mean total lengths of all laterals per plant on healthy roots only in a horizontal 6-inch section 6 to 12 inches below the surface. In one comparison the difference was 7.2 times the probable error, and in the other comparison, 7.0 times the probable error.

The significance of such differences in root systems is still further emphasized by the fact that plants of the strains susceptible to rootrot were considerably taller than plants of the good strain. The importance of such differences in ratio of lengths of roots which function to those of parts above ground is plainly evident.

Additional data on the relation of extent of root system to pulling resistance and lodging in the good strain and the strain susceptible to rootrot are presented in Table VIII. The difference of 6, or 16.2 per cent, in number of main roots, although not large, was very consistent and significant. Differences both in mean total number of lateral roots and mean total length of lateral roots were very marked. The strain susceptible to rootrot had a lower pulling resistance and more leaning plants than the good strain.

TABLE VIII.—*Data from a comparative study of plants from a good inbred strain and plants from an unrelated inbred strain susceptible to rootrot, the corn being planted May 22, 1922, in adjacent rows, near Bloomington, Ill., on brown silt loam soil on which the three previous crops were corn, 10 plants from each strain being excavated September 16–18, and 17 erect plants with green stalks from each strain being pulled September 28 and 29*

Character of strain.	Percentage of plants leaning 30° or more in a period of six years.						Mean resistance per plant to a vertical pull.	Mean number of main roots per plant.	Mean total number of lateral roots per plant on six 6-inch pieces of main roots 6 to 12 inches below surface.	Mean total length of lateral roots per plant on six 6-inch pieces of main roots 6 to 12 inches below surface.
	1917	1918	1919	1920	1921	1922				
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Pounds.*			*Inches.*
Good	0.0	8.9	0.0	0.0	7.8	2.5	251.5	37	318.4	434.5
Susceptible to rootrot	0.0	7.1	31.8	10.1	83.3	20.0	198.5	31	212.7	217.4
Difference between good and susceptible strains	....	....	....	....	....	....	53.0	6	105.7	217.1
Percentage difference based on good strain	....	....	....	....	....	....	21.1	16.2	33.2	50.0
Odds	....	....	....	....	....	....	322:1	3,332:1	554:1	908:1

HISTOLOGICAL STUDIES [5]

After it became known that there were marked differences in the extent and gross structure between the root systems of the good strain and the strain susceptible to rootrot, material was prepared to determine whether there was also any marked difference in their cellular structures.

Cross sections were made from the roots of six plants of each strain. All the preparations from each strain showed very similar cellular structures; in fact sections from any two plants were as much alike as sections from two roots of the same plant. But where sections of the two strains were compared a decided difference was noted. (Pl. 4 and 5.) The number of tracheids per root in the two strains was the same, but in the good strain they were large and oval in cross section, while in the strain susceptible to rootrot they were considerably smaller and round. The most important difference, however, lay in the cell arrangements of the pith and cortex. The cells of the good strain were closely united so that the cells had an angular shape. The corners were usually reinforced by extra thickenings. In the strain susceptible to rootrot these cells were round and were not closely bound together, neither did they have any thickenings in the spaces between the cells. Measurements of the cell walls of the pith and cortex also showed that the thickness of the cell walls of this strain was slightly less than that of the good strain, regardless of the special reinforcements possessed by the cells of the latter. Thus these preliminary studies indicate that there is a considerable difference between the roots of these two strains in the morphology of the cells themselves, which may account for some of the desirable qualities of the good strain.

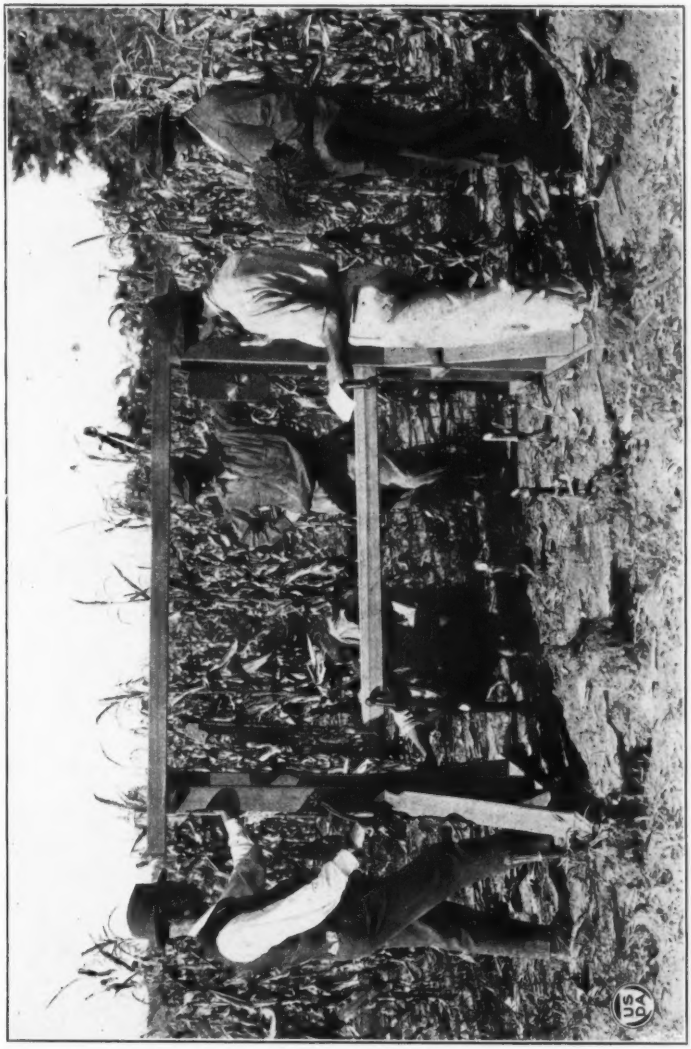
CONCLUSION

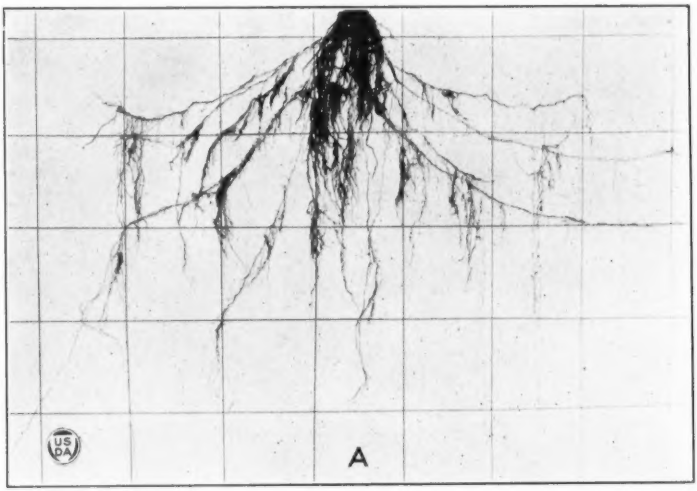
From the foregoing data it is evident that extent and distribution of root systems are very important factors in determining the economic importance of different strains of corn and their adaptability to various conditions.

[5] Acknowledgment is made to Prof. F. E. Wood and Dr. R. Hayes of the Illinois Wesleyan University for assistance and use of equipment in preparing the histological material.

PLATE 1

The root-pulling machine in operation. The corn stalks were first cut off at a convenient height. The upright standard next to the scale is open in the center at the bottom so that the corn stumps pass through it and the machine need not be lifted over them. The scale used was a Chatillon spring balance graduated in tenths of pounds with a capacity of 120 pounds at the scale. The maximum pulling resistance measured so far was 865 pounds. The scale is hooked to the beam by means of a chain so that it can be hooked at any height which will bring the beam into a horizontal position when the greatest strain is being exerted.

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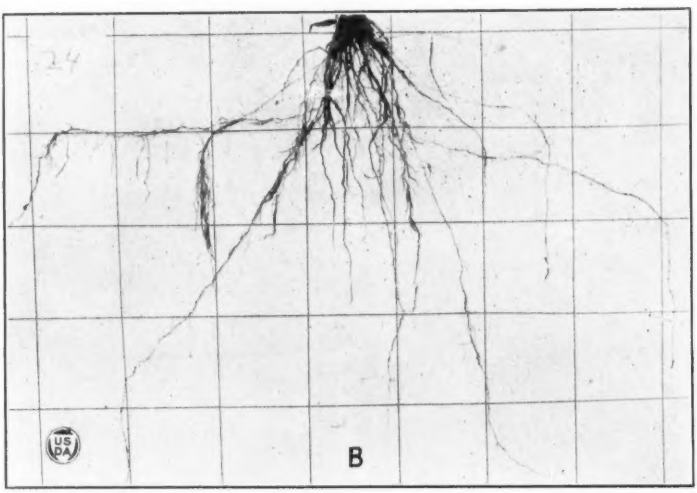
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PLATE 2

A.—Representative root system of the good strain of corn. The corn was planted June 2, 1922, and excavated when the plant was 46 days old. Previous to photographing, the roots were mounted on a frame with wires stretched at right angles 6 inches apart each way. On the frame the main roots were placed, as nearly as possible, in their original position in relation to a vertical line passing through the center of the base of the plant.

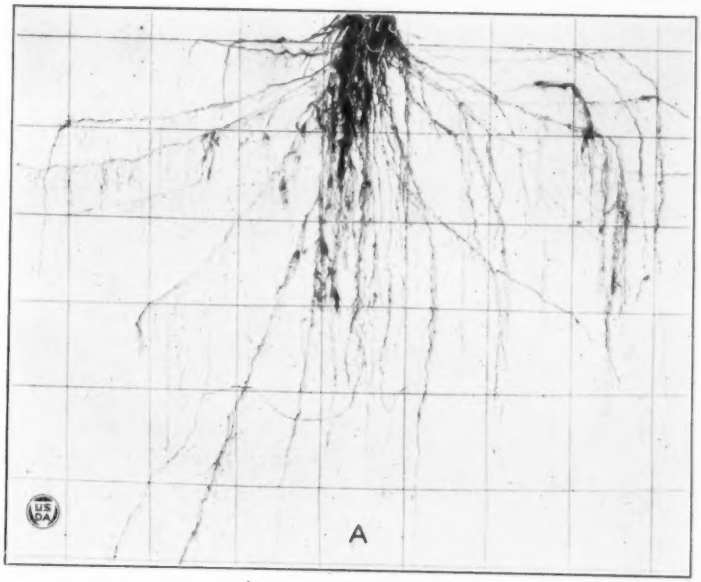
B.—Representative root system of a strain of corn susceptible to leaf firing. The corn was planted June 2 in a row adjacent to the good strain, and excavated when the plant was 46 days old. At the time the excavations were made the leaves of the strain susceptible to leaf firing were beginning to wilt, although there was no evidence of root rotting. Other similar plants of this same strain partially recovered after rains that broke the drought.

The differences in the two types of root systems are very marked, both in number of main roots and number and length of lateral branches.

PLATE 3

A.—Representative root system of a good inbred strain of corn. The corn was planted June 2, 1922, and was excavated 77 days after planting. Less than 10 per cent of the roots showed evidence of root rotting.

B.—Representative root system of an inbred strain of corn susceptible to rootrot. Over 96 per cent of the roots showed evidence of root rotting.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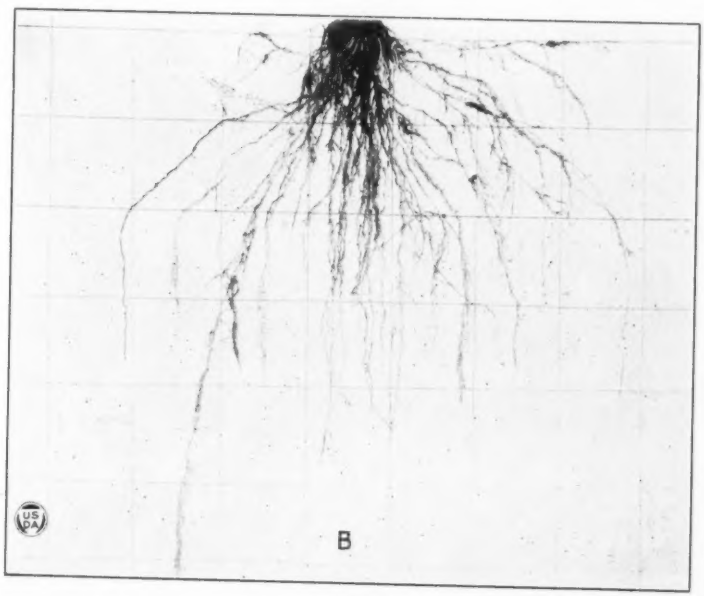
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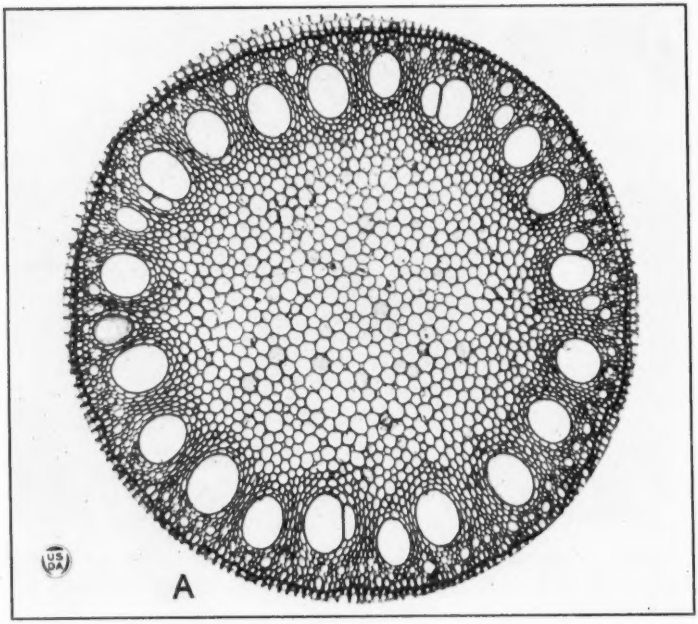
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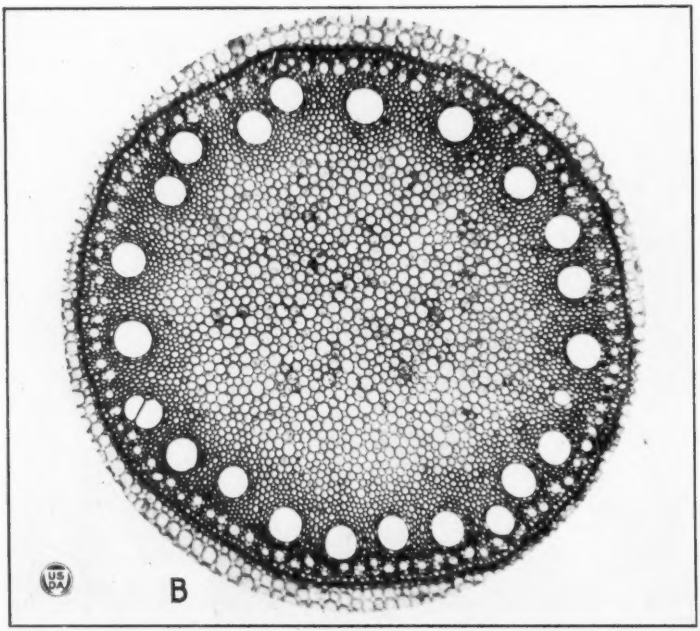
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PLATE 4

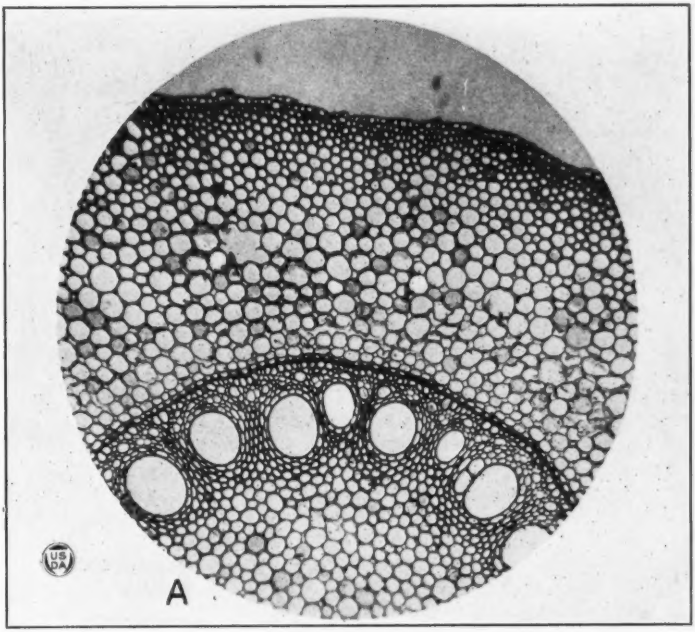
A.—Cross section of central stele of the root of a good inbred strain of Yellow Dent corn that was unusually vigorous, had no chlorophyll deficiencies, yielded well, and was highly resistant to rootrot and smut. The tracheids are large and the pith cells are closely bound together with thickenings at the corners.

B.—Cross section of central stele of the root of an inbred strain of Yellow Dent corn that possessed the other desirable qualities mentioned above but was susceptible to rootrot and lodged easily. The tracheids are comparatively small and the pith cells are very loosely connected so that they are round in cross section, whereas those above are angular. The spaces between the cells are open, and there are no special thickenings.

PLATE 5

A.—Cross section of the cortex of a good inbred strain, the same as shown in Plate 4, A. The cells are thick walled and are closely bound together, which gives the cells an angular shape in cross section. Many of the corners are reinforced by special thickenings.

B.—Cross section of the cortex of an inbred strain which is susceptible to rootrot, the same as shown in Plate 4, B. The cells are not as thick walled as those above; they are round in cross section, and are not closely bound together.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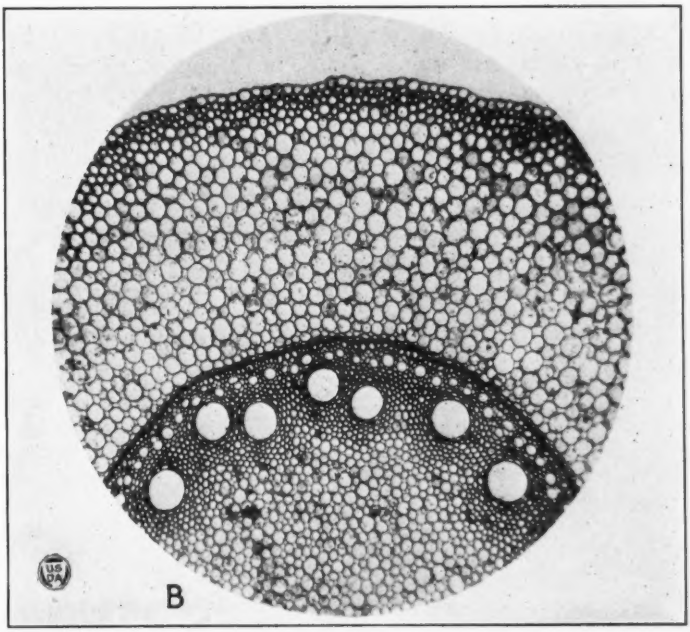
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ADJUSTING YIELDS TO THEIR REGRESSION ON A MOVING AVERAGE, AS A MEANS OF CORRECTING FOR SOIL HETEROGENEITY [1]

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GENERAL CONSIDERATIONS

The recent tendency has been to eliminate the use of check plats as a means of correcting for soil heterogeneity in comparisons of varieties or selections under field conditions. Thus, the committee on standardization of field experiments of the American Society of Agronomy reports:[2] "*Check plats.*—Adequate replication of varieties or treatments removes the necessity of including check plats."

The author is in entire accord with this view if the word "check" is used in the ordinary sense and "adequate" is defined properly. It is felt, however, that replication adequate to remove the advisability of having some measure of the variation in soil productivity from point to point is impractical under many conditions. Moreover, reducing the variability by doubling the number of replications does not necessarily make the data obtained as significant as if the same reduction were achieved by adjusting the yields to a check occurring in alternate plats. In the author's experiments with corn, therefore, the frequency of the checks has been increased, when possible, until they occurred in alternate plats and at the same time the number of replications has been kept as large as conditions permitted. For any fixed experimental facilities the size of the plats, the number of replications, and the frequency of check plats are the elements that determine the number of strains that can be compared. If corrections for soil heterogeneity could be made without the use of check plats, a material gain in accuracy should be possible.

With this in mind, a planting arrangement was devised for an experiment in which each of the strains to be compared was used as a check in one series of replications, thereby constituting that many extra replications of each strain. The data obtained under this arrangement were fairly satisfactory as a whole, but the method was limited in its application and there were other objections to it. Some of the concepts on which it was based were not fundamental and modification of certain phases made it more satisfactory and permitted a wider application. As some of the principles involved appear not to have been used in connection with varietal and strain comparisons, and appear to promise something of value, it seems advisable to present them at this time.

Before considering the method itself, it is desirable to discuss briefly the principles upon which it is based. This can be done more conveniently in connection with certain hypothetical experiments, the object of which is to determine the relative productiveness of two varieties or strains of corn in a given field for a single season. In these experiments

[1] Accepted for publication Nov. 19, 1923.

[2] WIANCKO, A. T., ARNY, A. C., and SALMON, S. C., report of committee on standardization of field experiments. *In* Jour. Amer. Soc. Agron., v 13, p. 372. 1921.

each variety is grown in 100 relatively long narrow plats arranged as shown below. The mean yields in each will be taken as 75 and 70 bushels per acre, respectively, with a probable error of ± 0.707 bushel in each case. It is assumed that errors due to differences in stand or to competition between varieties do not enter into the experiments. The planting arrangement in each case follows:

Experiment 1. The ends of the plats of one variety abut upon the ends of the plats of the other.

Experiment 2. The plats of the two varieties alternate.

Experiment 3. The two varieties are included among 100 in a varietal comparison, all of the varieties being replicated 100 times, with the replications systematically distributed.

THE SIGNIFICANCE OF THE PROBABLE ERROR

The significance of the data in relation to the probable error may be considered first. In each experiment the difference between the yields of the two varieties is 5 ± 1 bushels, and the odds are more than 1,300 to 1 that this difference is not due to chance. The presumption as to causal relations, however, differs markedly in each case. Thus, in experiment 1, one would hardly care to attribute the difference in yield to a varietal difference, whereas in experiment 2, such an assumption is entirely warranted. It is clear that the probable error in either case measures the same thing. The difference in the presumption as to the causal relations rests, of course, upon a knowledge that the yields of both varieties, in so far as they are affected by soil productivity, represent random samples of essentially the same soil mass in Experiment 2, but not in Experiment 1.[3]

The conditions in Experiment 3 do not permit such definite conclusions as to causal relations. The probable error again measures the portion of the difference that reasonably may be assigned to variations in the random sampling of the varieties and the soils in which they grew. Here, however, the yields as influenced by soil productivity do not sample entirely different soil masses, as in Experiment 1, nor do they sample what is essentially the same soil mass as was the case in Experiment 2. In so far as it is probable that 100 plats of one variety distributed over a 30-acre field[4] would not encounter soil conditions materially different, as an average, from those encountered by 100 plats of another variety distributed over the same field, it is logical to assume that the difference in this experiment was due to a difference in the varieties. Such an assumption is based, however, upon the judgment as to equal chances, and not upon the probable error per se.

The above consideration has been made somewhat detailed, not because it presents a new concept, but to emphasize clearly and distinctly that interpretation as to causal relations in field experiments expressed in terms of the probable error really involves two elements of judgment. These are (1) the probable reliability of the two means from the standpoint of the random sampling of the varieties and the soil in which each grew and (2) the conclusion that there was no systematic

[3] It is conceivable that a difference obtained as in Experiment 2 might be due to something other than an inherent varietal difference, such as some previous fertilizer or cultural treatment that was similarly alternated and the alternations of which chanced to coincide with those of Experiment 2. The possibility of such an occurrence without the knowledge of the investigator, however, scarcely needs to be considered. A more probable cause of error in the assignment of causal relations would be a difference in seed value not inherent in the varieties. The consideration of this kind of error is entirely beyond the scope of the present paper.

[4] Something over 30 acres would be required for 100 replications of 100 varieties where each plat consisted of 10 hills 3.67 feet apart.

difference in the conditions of soil productivity under which the two varieties were grown. When the number of replications is large in proportion to the number of strains compared, the correctness of the latter conclusion obviously may be warranted. As the number of replications decreases relatively to the number of strains involved, however, the justification for such a conclusion becomes constantly less. Thus, when 100 strains are systematically replicated 4 times, any 2 strains may not have been grown within 10 plats of each other in the whole experiment.

THE COEFFICIENT OF CORRELATION

When the relation between two variables is rectilinear, the coefficient of correlation measures their tendency to concomitant variation. A significant coefficient of correlation shows a relation between the variables, but tells nothing of the cause of that relation. One may be the cause or effect of the other, the relation may be purely mathematical, as between a product and its factors, or both variables may be affected by one or more common causes. Frequently, however, other information is available on which to base interpretations. Thus, a positive correlation between the yields of the adjacent odd and even rows in Experiment 2 logically leads to the conclusion that the yields of both varieties were being influenced from row to row by variation in the soil conditions so gradual that when it affected one row, it also affected the adjacent row more or less. Similarly, systematic competition would tend to lower a positive correlation due to soil variation, and might even result in a negative correlation.

For a good discussion of the interpretation of data on the basis of correlation values plus such other information as to the relations as may be available, the reader is referred to the works of Sewall Wright.[5, 6]

Letting X and Y represent the odd and even rows of Experiment 2, the significance of the coefficient of correlation is made more evident by its relation to the standard deviation of the differences. Thus, the standard deviation of the differences, $X-Y$, is given by the equation, $\sigma^2_{X-Y}=\sigma^2_X+\sigma^2_Y-2r_{XY}\,\sigma_X\,\sigma_Y$. When $r=0$, $\sigma^2_{X-Y}=\sigma^2_X+\sigma^2_Y$. Substituting probable errors for standard deviations, the latter is the equation largely used for the probable error of a difference between means. This is correct in field experiments only when there is no correlation between the yields of the two items in the different replicates. It results in too small an error if there is a negative correlation and too large an error if r is positive. Competition thus tends to lower, and a gradual change in soil productivity tends to raise, the probable error computed in this way. It is the portion of the error due to the term, $2r_{XY}\,\sigma_X\,\sigma_Y$, in the formula for the squared standard deviation that is eliminated by obtaining the successive differences between X and Y and determining their probable error directly.

REGRESSION

It is in connection with the theory of regression that the coefficient of correlation attains its importance in the present paper. Using X and Y as in the previous paragraph, the regression of X on Y is given by the expression, $r_{XY}\,\frac{\sigma_X}{\sigma_Y}$. In other words, for each unit of deviation of Y from

[5] WRIGHT, SEWALL. CORRELATION AND CAUSATION. *In* Jour. Agr. Research, v. 20, p. 557-585. 1921. Literature cited, p. 585.

[6] ———. THE THEORY OF PATH COEFFICIENTS. *In* Genetics, v. 8, p. 239-255, 8 fig. 1923. Literature cited, p. 255.

its mean, X on an average deviates $r_{XY}\frac{\sigma_X}{\sigma_Y}$ times one unit, from its mean. The deviation is in the same direction if the regression is positive ($r=+$), or in the opposite direction if it is negative. Similarly, $reg_{YX}=r_{XY}\frac{\sigma_Y}{\sigma_X}$. Thus, when any row X deviates from its mean by x units, the magnitude $x\left(r_{XY}\frac{\sigma_Y}{\sigma_X}\right)$ is the predicted deviation of the associated Y row from its mean. Letting the actual deviations of rows Y from their mean be represented by y and the predicted deviations by y_1, the y_1's are the closest estimates of the y's that it is possible to obtain, knowing the x's and assuming a rectilinear relationship.

Let $y-y_1=y_2$, the error of estimate or residual. There are, of course, N y_2's in N paired observations. Their mean is zero and their standard deviation is given by the equation $\sigma_{y_2}=\sigma_Y\sqrt{1-r^2_{XY}}$. This is the "standard error of estimate," and its value is less for the y_1's than for any similar values predicted on the basis of x.

ADJUSTING YIELDS TO CHECKS

The assumption underlying the methods that have been generally used in adjusting the yields of test plats to those of nearby checks has been equivalent in effect to assuming an entirely arbitrary correlation between the two kinds of plats. Let c_1 and c_2 be the yields of two adjacent checks, C the mean yield of all checks, and Y and Y_a the actual and adjusted yields of the test. Then, adjusting for the checks on each side according to the equation, $\frac{2Y}{c_1+c_2}C=Y_a$, is equivalent to adjusting on the basis of an assumed regression coefficient of $+1$. When only every third or fifth plat is a check and the regression is supposed to decrease proportionately with the distance from the checks, the assumption is even more arbitrary. That such methods of adjustment frequently have reduced the variability of the test plats materially is ample evidence of the fundamental soundness of some means of correcting for soil heterogeneity.

Without a detailed review of the literature, Stadler's [7] discussion of the value and limitations of adjusting to checks may be cited as approximating in general the conclusions of other investigators. These may be summed up briefly in the statement that adjusting sometimes is beneficial and sometimes it is not. Stadler also discusses some of the conditions under which adjustment was more and less effective in his experiments. These may be grouped into conditions that would tend to increase and those that would tend to decrease the significant correlation between the check and the test plats. It is clear that the practice of adjusting to check plats is unwarranted when there is no correlation between the yields of the tests and the checks. It is equally clear that an adjustment to the checks on the basis of the actual regression of the test plats on the check plats is warranted and of value. On this basis it would be desirable to have frequent check plats in every experiment and to use them for adjusting the yields only in those cases in which there was a significant correlation between their yields and those of the test plats. Because of the area required, this is not practical unless the

[7] STADLER, L. J. EXPERIMENTS IN FIELD PLOT TECHNIC FOR THE PRELIMINARY DETERMINATION OF COMPARATIVE YIELDS IN THE SMALL GRAINS. Mo. Agr. Exp. Sta. Research Bul. 49, p. 72, 1921.

"check plats" also constitute replications. The planting arrangement to be described was an effort to obtain this result.

THE METHOD USED

THE PLANTING ARRANGEMENT

The planting arrangement was used in connection with corn breeding experiments conducted by the Office of Cereal Investigations of the United States Department of Agriculture, at Burdette, Ark., in 1921. The detailed data have been published,[8] and need not be repeated. It should be noted, however, that the field in which this experiment was located was so lacking in uniformity that it was used only because of circumstances amounting practically to necessity.

The planting arrangement and methods of computation are indicated in Table 1, which gives the order of planting for the first 10 rows of series 1 to 5. The different lots are referred to by the strain numbers given in Table 2.

TABLE I.—*Abstract of data from the comparison of generations in a selection experiment at Burdette, Ark., in 1921, illustrating the order of planting and the method of computing the percentage yields*

Row No.	Series 1.							Series 2 to 5.			
	Strain number.	Actual yields, pounds.[a]			Percentage yields.			Strain numbers in series:			
		Individual rows.	2-alternate-row plats.	3-row plats.	Individual rows.	2-alternate-row plats.	3-row plats.	2	3	4	5
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
1	10	9.8	.....	.....	[b] 95.1	.....	.....	10	10	10	10
2	1	14.4	21.2	35.6	[b] 113.3	[b] 92.1	[b] 99.7	2	3	4	5
3	1	11.4	27.0	38.4	89.7	106.2	100.7	1	1	1	1
4	1	12.6	23.8	36.4	99.2	100.0	99.7	2	3	4	5
5	2	12.4	28.0	40.4	111.7	110.2	110.6	2	2	2	2
6	1	15.4	26.5	41.9	121.2	112.2	115.4	2	3	4	5
7	3	14.1	25.6	39.7	112.8	100.7	104.7	3	3	3	3
8	1	10.2	24.0	34.2	80.3	104.8	96.0	2	3	4	5
9	11	9.9	20.8	30.7	95.1	81.8	85.7	11	11	11	11
10	1	10.6	20.5	31.1	83.4	89.5	87.3	2	3	4	5

[a] On basis of corrected stand. [b] Not used in correlations as there were no comparable 3-row plats.

Each strain was grown in a single row between one-row check plats. The rows were 10 hills long with two plants in each hill. A single comparison of all of the strains with the alternating check plats constituted a series which was replicated 10 times. A different strain, however, was used as the check in each unit. Thus, strain No. 1 was the check in the first unit or series, strain No. 2 was the check in series 2, and so on, each of the seed classes, strains 1 to 10, inclusive, being used as a check in one of the series. Series Nos. 1 to 5, inclusive, were grown end to end, and series 6 to 10 were adjacent to Nos. 1 to 5. A test plat of strain No. 11 was planted in each of the 10 series, but this strain was not used as a check.

[8] RICHEY, F. D. EFFECTS OF SELECTION ON THE YIELD OF A CROSS BETWEEN VARIETIES OF CORN U. S. Dept. Agr. Bul. 1209. (In press.)

The use of the small plats, containing approximately 0.0029 of an acre, made it possible to have a large number of replications of each strain. Twelve of these replications were distributed in alternate rows across one series and 10 were distributed lengthwise of the experiment. The strain occurring in alternate rows in any one series served as a check on soil variation within that unit and the strains as a whole distributed lengthwise of the field served as controls on differences between the series. Looked at in this way, therefore, there were alternate check plats throughout the experiment without any increase being required in total area. Moreover, each strain competed with every other strain the same number of times.

TABLE II.—*The mean actual and adjusted yields and the average values of the corresponding 3-row plats of 11 strains of corn compared at Burdette, Ark., in 1921*

Strain No.	Designation.	Average of 22 replications.[a]				
		Actual yields.		Adjusted yields, basis of—		Average of 3-row plats.
				2-alternate-row plats.	3-row plats.	
1	*2*	*Pounds.* *3*	*Bushels.* *4*	*Bushels.* *5*	*Bushels.* *6*	*Per cent.* *7*
1	Whatley Prolific.....	12.7	73.48±1.50	72.61±1.21	72.66±0.76	101.21
2	St. Charles White.....	11.1	64.22±1.45	63.99± .98	63.96± .73	100.88
3	St. Charles White × Whatley, F_1.....	12.5	72.32±1.45	73.25± .98	72.85± .67	99.18
4	Whatley × St. Charles White, F_1[b].	12.5	72.32±2.43	72.90±1.56	72.45± .99	99.32
5	Selection No. 201 F_2..	11.2	64.80±1.62	67.81±1.04	66.77± .72	97.78
6	Selection No. 201 F_3..	10.2	59.01±1.16	56.35±1.04	56.94± .75	103.44
7	Selection No. 201 F_4..	11.5	66.54±1.62	68.73±1.27	67.69± .80	98.10
8	Selection No. 201 F_5..	11.4	65.96±1.21	64.51±1.04	64.80± .72	101.75
9	Selection No. 201 F_6..	11.5	66.54±1.68	66.88±1.10	66.59± .74	100.02
10	Selection No. 201 check.....	10.3	59.59±1.68	58.49±1.21	58.65± .77	101.41
11	Selection No. 201 F_1 (1914 seed).....	10.4	60.17±3.53	64.11±2.72	62.43±1.92	94.73
	Average of the probable errors[c]	.....	1.757	1.287	.870	[d]100.07

[a] Only 10 replications of strain No. 11.

[b] Selection No. 201 is the cross, Whatley × St. Charles White, made in 1914 and the filial generations descended from it.

[c] P. E.$=0.6745\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

[d] Average of all 3-row plats.

ADJUSTING THE YIELDS

The mean yield of each strain was computed first. These means are given under the heading, "Actual yields" in columns 3 and 4 of Table II. As has been noted, the field in which the comparison was made did not even look uniform, and, although the different lots were well distributed over the area, it was uncertain whether they had equal opportunity. It was assumed that the ratio of the yield of two alternate rows to the mean yield of the strains grown in them would be a good index of the productivity of the soil between them. Accordingly, the yield of each row was adjusted to this ratio. Thus, the yield of 15.4 pounds for row

6, series 1 (Table I) was divided by 1.122, the ratio of the yield of rows 5 and 7 (26.5 pounds) to the sum of the average yields of strains 2 and 3 for the entire experiment (23.6 pounds) which were grown in rows 5 and 7 of series 1. The yield of each row was adjusted similarly and the adjusted yields then were averaged. The means are given under the heading "Adjusted yields, basis of 2-alternate-row plats" in column 5 of Table II, for comparison with the actual yields.

EFFECTS OF CORRECTION

Inspection of Table II shows that all of the means have been modified one way or the other. Many of the modifications are so slight as to be entirely negligible, whereas others appear to have some significance. There is a consistent difference, however, in the probable errors of the two sets of means, those of the relative yields being lower in every case. The mean of the probable errors for the actual yields, 1.757 bushels, is reduced to 1.287 for the relative yields, a reduction of 0.47 bushel or 26.75 per cent. This represents a real gain in the degree of accuracy with which comparisons can be made between the strains.

As will be shown later, the assumption of a 1:1 ratio between the variations in yield of the plats of two alternate rows and that of the row between them was not warranted entirely. It was sufficiently accurate, however, to eliminate one-fourth of the variation as evidenced by the smaller probable errors.

EXTENSION OF METHOD

As previously noted, the method used was not entirely satisfactory. In the first place, the extreme variability of the soil made the use of such short rows questionable. These conditions combined to make the plat yields fluctuate rather widely with the result that sometimes a yield, actually too low as judged by its ratio to the mean for the strain, was decreased still further because the adjacent rows yielded more than their mean. Part of this may have been due to competition, but much of it was a result of random fluctuation. Finally, a planting arrangement in which the replicates were distributed more uniformly would have been better. Objections inherent in the calculations are subject to elimination. Other methods, therefore, were tried, and their effects noted.

OTHER BASES FOR ADJUSTING

If the mean actual yields of the different strains represented approximately their respective productiveness, it was reasonable to assume that the ratio of the actual yield of a plat of any given number of rows to the computed average yield of a group of rows of equal number and kind would be an approximate index of the productivity of the soil in which the given plat was grown. On this hypothesis, indices of productiveness were computed for plats of three and five adjacent rows, in addition to those for the plats of two alternate rows already considered. In order to make the indices continuous, the moving average commonly used in time series was utilized. The method of computation may be indicated from the data in Table I for the 3-row plats. Thus, the 3-row index (column 8) centered on row 7, series 1, was obtained by dividing the total yield of 39.7 pounds (column 5) for rows 6 to 8, inclusive, by 37.9 pounds, the total yield of two average rows of Strain No. 1 plus one average row of Strain No. 3 (Table II, column 3).

The quotient, 104.7 per cent, is the percentage yield, or index of productiveness for a plat centered on row 7. It should be noted that these indices were not computed by averaging the percentage yields of the individual rows.

THE EFFICIENCY OF THE DIFFERENT CHECKS

The efficiency of the plats of different sizes as a basis for adjustment was studied through their correlation and regression relations to the percentage yields of the individual rows, and through the reduction in the variability of the latter which was accomplished by adjusting to the different kinds of checks.

For convenience, the individual rows will be considered as variable A, the plats of two alternate rows as variable B, and the 3 and 5 row plats as T and C, respectively. The constants reported for these plats were computed from correlation tables in which the class interval was 5 per cent with class centers at . . . 95.05, 100.05, 105.05, etc. The table for r_{AT} is shown in figure 2.

The coefficients of correlation of A with B, T, and C, and the regression of A on these variables, the gross standard deviations of the different kinds of plats, and the net standard deviations of A for constant B, T, and C are shown in Table III. All of these indicate the superiority of the 3-row moving average for adjusting yields. The net standard deviation of A with one variable constant is the same as the standard error of estimate for the coefficients of regression of A on that variable and shows the amount of variation that will remain in A after adjusting it on the basis of that regression. The relative fit of the 3 and 5 row plats to the individual rows for series 1 and 6 and series 2 and 7 is shown graphically in figure 1. The fit of the plats of two alternate rows is not shown, but may be estimated readily from the individual row values.

The reliability of the correlation coefficients and the constants derived from them rests upon the assumption that the relations between the variables are rectilinear. That the latter assumption is warranted as regards the relations of the individual rows with the 3-row plats is shown clearly in figure 2, in which the means of A for the corresponding values of T are shown by the plus sign (+). The regression line for A on T also is shown in figure 2.

TABLE III.—*Coefficients of correlation of A with B, T, and C; coefficients of regression of A on B, T, and C; gross standard deviations, in percentages, of A, B, T, and C; and partial standard deviations of A for constant B, T, and C, for a single row and for the mean of 4, 10, and 22 rows*

Variable.	Coefficients of—		Percentage σ of variable in column 1.	Percentage σ_A for the mean of the number of rows stated, when the variable in column 1 is made constant.			
	Correlation, A with variable in column 1.	Regression A on variable in column 1.		1 row.	4 rows.	10 rows.	22 rows.
1	2	3	4	5	6	7	8
A	.....	.....	16.805	.....	[a] 8.403	[a] 5.315	[a] 3.583
B	0.6995±0.0230	0.830	14.170	12.009	6.005	3.798	2.560
T	.8616± .0116	1.027	14.100	8.530	4.265	2.698	1.819
C	.8266± .0143	1.117	12.440	9.458	4.729	2.992	2.016

[a] Gross σ_A.

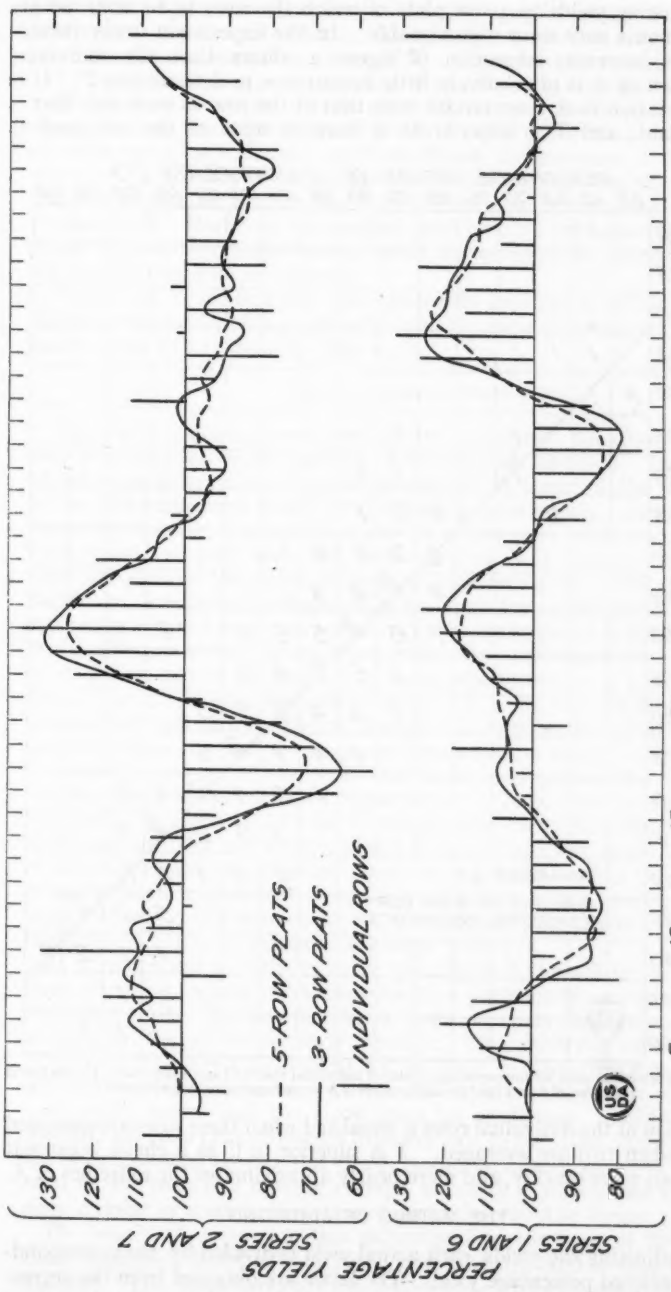

FIG. 2.—Graph showing the percentage yields of individual rows, 3-row plats, and 5-row plats in series 1 and 6, and 2 and 7.

Adjusting yields to 3-row plats of which the rows to be adjusted are components may seem objectionable. In the experiment under consideration, however, inspection of figure 1 shows that the individual variation of A is of relatively little importance in determining T. It is the variation in A concomitant with that of the row on each side that is important, and T is superior to B because more of the independent

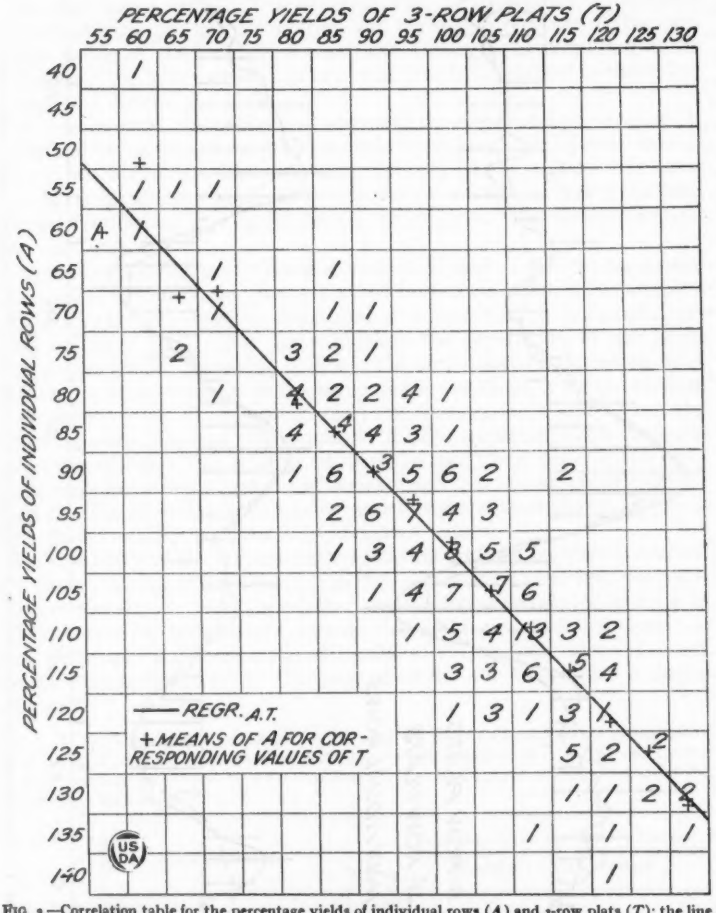
PERCENTAGE YIELDS OF 3-ROW PLATS (T)
55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130
PERCENTAGE YIELDS OF INDIVIDUAL ROWS (A)
40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140
REGR. A.T.
+MEANS OF A FOR CORRESPONDING VALUES OF T
USDA

FIG. 1.—Correlation table for the percentage yields of individual rows (A) and 3-row plats (T); the line of regression of A on T; and the mean values of A for each corresponding value of T.

variation of the individual rows is equalized when three rows are averaged than when two are averaged. T is superior to C as a check because it responds more quickly, and more nearly approximates the extremes of A.

THE METHOD OF ADJUSTING

In adjusting the yields, each actual yield is divided by the corresponding predicted percentage yield. The latter are obtained from the regression equation, in this case, $a = 1.027t$, in which a and t are the deviations

from their respective means, or this equation may be converted to absolute values, $A = 1.027\ T - 0.027$. The predicted A's then may be calculated for the corresponding values of T. Thus, for row 6, series 1, $T =$ 115.4 per cent, or 1.154. $1.154 \times 1.027 = 1.185$, $1.185 - 0.027 = 1.158$, the predicted yield of row 6. The actual yield, 15.4 pounds, divided by 1.158 = 13.3 pounds, the adjusted yield of row 6. The "Adjusted yields, basis of 3-row plats" in column 6 of Table II are the averages of the yields corrected in this way. Here again the means are modified slightly, but the gain in reliability as measured by the decrease in the probable errors is about half. Moreover, the method used here is based upon the determined regression and involves no arbitrary assumption, which was not the case for the data in column 5.

In practice it will be easier and sufficiently accurate to obtain the predicted percentage yields graphically from the regression line drawn as in figure 2, but to a reasonably large scale.

OTHER CONSIDERATIONS

The planting arrangement used in the experiment described is in no way fundamental to the method of adjustment proposed. In fact, it would have been better from the standpoint of theory if the replicates of the different items had been distributed more widely. Neither is it necessary to have as many replicates as in this case. It is necessary to have plats of such size and frequency as will give a reasonable approximation of the productiveness of each seed class. The replicates should be distributed systematically to cover the experimental field as uniformly as possible, and in such a way that the sequence is not the same in the different series. Thus, representing the seed classes by 1, 2, 3, 4, etc., the first may be planted 1, 2, 3, 4, etc., the second, 1, 3, 5, 7, etc., the third, 1, 4, 7, 10, etc., and the fourth, 1, 5, 9, 13, etc., or any similar system that will give a different sequence in each series. The index of productiveness for each 5-row group under such a system would be in terms of the average of 20 rows located in different parts of the field, and the final mean of four adjusted replicates would be weighted according to the yield of as many as 80 rows in a large experiment properly arranged. Moreover, although not all strains will be used equally in correcting each of the others unless the number of replications is the same as the number of strains in the experiment, nevertheless the basis will be an average of a large number of strains in each case, thus reducing the chances of distortion due to the specific response of a single variety.

It is recognized that the extreme variability of the soil in the experiment discussed in some ways made the data particularly amenable to the correction used. On the other hand, probably many experimental fields are equally, though not similarly, variable. The rapidity of the variation in the case considered, as brought out in figure 1, made conditions decidedly adverse for adjustment. It was this rapid variation that made the 3-row plats better than the 5-row plats for predicting. A field with a gradual change in productiveness from one side to the other, but with negligible fluctuations around this trend, would be ideally suited to adjustment on the basis of a moving average, although in such a case the optimum number of rows to be averaged probably would be larger.

The important points are that adjustment on the basis of the determined regression is not arbitrary and that no extra land or field labor is required. The computations of the indices and correlation are simple, and,

if the latter is not significant, no adjustment of individual yields need be made. The following values show the average percentage reduction in the probable errors resulting from adjustment when the correlation between tests and checks is as stated:

$r=$	Reduction in variability $=100\times 1-\sqrt{1-r^2}$	$r=$	Reduction in variability $=100\times 1-\sqrt{1-r^2}$
	Per cent.		*Per cent.*
0.4	8.4	0.8	40.0
.5	13.4	.866	50.0
.6	20.0	.9	56.4
.707	29.3	1.0	100.0

It is evident that correlations of less than 0.6 will reduce the variability so little that adjustment of individual yields may hardly be worth while.

In the case of relatively low correlations, however, the moving average may be used as a criterion in another way. Individual yields may be modified by adjusting so as to change the mean as well as the standard deviation. Thus, if one strain occurred only on relatively poor soil in each of four replications, the four yields might be identical, but all relatively too low. This fact should be brought out by the average of the indices of productiveness for the four soils in which this strain was grown. Therefore, if individual adjustments are not made, the averages of the indices may be obtained and taken into account in drawing conclusions from the data. As an example of this use, the average percentage values of the 3-row checks for each of the strain numbers are given in Table III, column 7. These seem to be a fair measure of the reliability of the comparison, and if the difference between two strains were materially greater than the difference between the corresponding indices more significance could be attached to it.

CONCLUSION

The fundamental concepts on which the proposed method is based are: (1) That the mean yield of any one seed class is a fair measure of its productiveness, (2) that the deviations of the individual replicates from this mean are due in part to soil variation, (3) that deviations of the yields of successive groups of contiguous rows from the computed production of an equal number of average rows of similar kind are due in part to soil variation, and (4) that the correlation of the percentage yields of such groups with the percentage yields of the individual rows upon which the groups are centered measures their tendency to concomitant response to the soil variation. Granted these, and they seem entirely sound, the adjustment of yields on the basis of their regression on a moving average follows logically.

SOYBEAN MOSAIC: SEED TRANSMISSION AND EFFECT ON YIELD[1]

By JAMES B. KENDRICK, *Assistant in Botany*, and MAX W. GARDNER, *Associate in Botany, Indiana Agricultural Experiment Station*[2]

INTRODUCTION

Rather extensive inoculation tests in field and greenhouse have failed to reveal any host for soybean mosaic other than the soybean itself. Cross inoculations from mosaic red clover and garden bean to soybean were unsuccessful, and cross inoculations from soybean to sixty varieties of garden beans and seven other species of Phaseolus, to two species of Dolichos, to field peas, and to cowpeas gave only negative results.

Mosaic has been noted on the following varieties of soybeans at La Fayette, Ind.: Midwest or Medium Yellow, Haberlandt, Manchu, Ito San, Mongol, Hurrelbrinks, Mammoth Black, Habara, A. K., Arlington, Hoosier, Elton, Wea, Lexington, Black Eyebrow, Pinpu, 36847, Feldun, Dunfield, Soysota, Wilson Black, Mammoth Yellow, Brown, Virginia, and Tar Heel Black. The disease seems to be most prevalent in the Midwest, Haberlandt, and Black Eyebrow varieties and the symptoms seem to be most conspicuous in the Midwest variety. The Midwest or Medium Yellow variety has been erroneously known locally as Hollybrook, according to Wiancko and Mulvey (*9*),[3] and it was in this variety that we (*5*) first noted the disease.

As yet the mosaic disease does not seem to have become seriously prevalent in Indiana. F. E. Robbins found the disease in only 4 out of 27 soybean fields inspected in 1923. With the exception of one field of the Midwest variety, only a very low percentage of infection has been found in commercial fields.

In addition to the typical mosaic symptoms described in a previous account by the authors (*5*), other symptoms have been found associated with the disease, but these have not been relied upon as criteria of mosaic in the following work. Among these symptoms are a bronzing of the young leaves produced by a brown discoloration of short segments of the veins and large splotches on the older leaves produced by a lacelike yellowing or browning of the veins. In the Lexington variety severe symptoms were evinced in 1923. The mosaic plants were extremely stunted, the growing tips were killed outright, and brown necrotic streaks developed on the stems and petioles.

SEED TRANSMISSION

In the previous account (*5*) it was recorded that about 13 per cent of the seed from mosaic plants transmitted the disease. Subsequent tests have given similar results with a number of varieties and with older seed.

The presence of the disease in ordinary commercial seed one year old was shown in 1921. Fifteen varieties and selections were planted in

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[3] Reference is made by number (italic) to "Literature cited," p. 98.

parallel plots of three rows each on May 26, 1921, and the occurrence of a very small percentage of seed-borne mosaic in 9 of the 15 varieties was determined by a careful examination of the seedlings on June 26 as shown in Table I. The secondary spread of infection as shown by the records of August 16 will be referred to later.

TABLE I.—*Mosaic in variety plot, 1921*

Variety.	Number of plants.	Mosaic seedlings June 26.	Mosaic plants, Aug. 16.	
			Number.	Per cent.
Midwest	272	0	79	29
Elton	253	2	24	9
36847	236	0	1	0.4
Haberlandt	255	5	57	22
Wea	294	0	28	9
Lexington	352	1	8	2
Soysota	221	0	0	0
A. K.	205	1	17	8
Black Eyebrow	160	1	21	13
Pinpu	209	0	7	3
Feldun	270	1	7	2
Dunfield	259	2	14	5
Ito San	250	0	4	2
Arlington	301	2	18	6
Manchu	166	1	12	7

In a small plot of Midwest soybeans planted in 1921 with seed from a field in which the disease occurred in 1920, 17 out of 423 seedlings, or 4 per cent, developed mosaic. A number of volunteer seedlings came up in 1921 in the field used in 1920 and one of these showed mosaic, indicating that the disease persisted over winter in the seed lying in the field.

Two rows were planted in 1921 with seed saved from mosaic Midwest plants in 1920 and out of the 156 seedlings, 14, or 9 per cent, showed mosaic. Among the progenies of six mosaic plants, 12 out of 65 seedlings, or 18 per cent, were mosaic.

In a plot planted in 1922 with seed saved from mosaic Midwest plants in 1921, 172 out of 993 seedlings, or 17 per cent, showed mosaic, while in another plot planted with seed from healthy plants, 590 seedlings came up and none were mosaic.

These results with seed from mosaic plants corroborate the writers' previous conclusion that a varying and usually rather low percentage of such seed carries the disease. Dickson (*3, p. 83–86*) obtained similar results in connection with the seed transmission of mosaic in *Trifolium pratense* L. and *Melilotus alba* Desr. Reddick and Stewart (*8*) found a varying but much higher percentage of transmission of bean mosaic through the seed, and Archibald (*1, p. 62*) found that 43 per cent of the seed from mosaic bean plants transmitted the disease. Doolittle and Gilbert (*4*) found that a low percentage of the seed from mosaic wild cucumber transmitted cucurbit mosaic, and Newhall (*6*) likewise found in the case of lettuce mosaic that a very low percentage of the seed transmitted the disease.

Seed saved from mosaic soybean plants of a number of varieties in the fall of 1921 were planted in the greenhouse the following winter with results as shown in Table II. The results of a similar test made with seed selected from mosaic plants in 1922 are shown in Table III.

TABLE II.—*Seed transmission by different varieties, 1921*

Variety.	Number of plants.	Number of seeds.	Number of seedlings.	Per cent germination.	Number of mosaic.	Per cent mosaic.
Midwest	10	676	535	79	121	23
Haberlandt	8	435	350	80	23	7
Black Eyebrow	1	31	23	74	8	35
Arlington	1	46	17	37	1	6
Feldun	1	151	122	88	4	3
Lexington	1	62	58	93	0	0
Dunfield	1	45	17	38	0	0
Manchu	1	64	27	42	0	0

TABLE III.—*Seed transmission by different varieties, 1922–23*

Tested.	Variety.	Number of plants.	Number of seedlings.	Number of mosaic.	Per cent mosaic.
In winter in greenhouse	Midwest	19	114	11	10
	Haberlandt	8	97	7	7
	A. K.	3	17	2	12
	Feldun	2	21	0	0
	Lexington	3	23	0	0
In summer of 1923 in field	Midwest	17	54	5	9
	Haberlandt	6	88	3	3
	A. K.	2	18	0	0
	Lexington	2	20	0	0
	Manchu	2	18	0	0

The results shown in Tables II and III indicate that varieties differ somewhat in their ability to transmit mosaic. The Midwest, Haberlandt, Black Eyebrow, A. K., and Arlington varieties apparently transmit the disease more readily than Feldun, Manchu, Lexington and Dunfield.

Individual plants of the same variety also differ greatly in the extent to which the disease is transmitted to their progeny. In the progenies of six single selections (Midwest) planted in the field in 1921, the percentage of mosaic varied from 0 to 33 per cent.

In the progenies of the ten single plant selections from mosaic Midwest plants in Table II, the percentage of mosaic seedlings varied from 6 to 38 per cent, and among the eight Haberlandt progenies the percentage of mosaic varied from 0 to 16. In the progenies of the 19 Midwest plants recorded in Table III, mosaic was absent in eight and varied from 7 to 50 per cent in the others. Four of the eight Haberlandt progenies also showed no mosaic.

To determine whether this peculiar incidence of mosaic had any relation to the node at which the pods were borne, the seeds from nine mosaic Midwest and seven mosaic Haberlandt plants were harvested separately by nodes in 1921 and tested in the greenhouse. No particular relation was found between the percentage of mosaic seedlings and the location of the node at which the seed was borne. Numbering the bearing nodes from the top down, the second, third, fourth, and seventh yielded the most mosaic seedlings in the Midwest plants and the fourth node in the Haberlandt plants. Numbering the bearing nodes from the base upward, the fourth, seventh, and eighth yielded the most mosaic in the Midwest plants.

That the variation in percentage of seed transmission of mosaic was not dependent upon the date of infection of the parent plant is indicated by the fact that in the 19 Midwest parent plants recorded in Table III the mosaic was of seed origin, and yet an average of only 10 per cent of mosaic occurred in the progenies of these plants; in fact, in eight progenies no mosaic occurred. In 4 of the 8 Haberlandt parent plants the mosaic was also of seed origin and the progenies of these plants showed a lower average percentage of mosaic than the progenies of the other four plants.

The effect of age of seed upon the transmission of mosaic is of practical interest. The field tests noted above proved that the disease persisted in the seed from one season to the next. Among 1,105 Haberlandt seedlings grown in the greenhouse in December, 1922, from commercial seed 15 months old, 3.2 per cent were mosaic. To test the effect of an extra year's storage, commercial seed from two of the same lots tested in 1921 (Haberlandt and Arlington, Table I) was planted in the field in 1922. Of the 560 Haberlandt seedlings, 7 were mosaic and of the 629 Arlington seedlings 2 were mosaic. It thus appears that the disease was present in 2-year-old seed.

That 2-year-old seed may carry the mosaic disease was further demonstrated by greenhouse tests in the spring of 1923 with seed harvested from single mosaic plants in 1921. The results, as shown in Table IV, indicate that there was a considerable percentage of mosaic transmission in the old seed from mosaic plants in the Black Eyebrow and Midwest varieties and a low percentage in the Haberlandt variety.

TABLE IV.—*Presence of mosaic in seed stored 16 months*

Plant.	Variety.	Per cent germination.	Number of seedlings.	Number of mosaic.	Per cent mosaic.
1	Haberlandt	77	88	3	3.4
2	Black Eyebrow	35	21	3	14.3
3	Wea	75	40	0	0
4	Lexington	77	31	0	0
5	Arlington	28	19	0	0
6	.....do	14	14	0	0
7	Midwest	64	38	26	68.4
8	.....do	98	46	3	6.5
9	.....do	69	22	6	27.2

In the summer of 1923, seed from six mosaic Midwest plants collected in 1921 was planted in the field and 36 of the 208 seedlings, or 12 per cent, came up showing mosaic. These results indicate that the use of 2-year-old seed can not be recommended as a mosaic control measure.

In 1921 and 1922 a conspicuous brown mottling of the seed coat occurred rather generally and occasioned considerable concern among the growers of soybeans for seed. As yet no relationship has been established between this seed mottling and the mosaic disease. Mottled seeds have been produced by both healthy and mosaic plants and in germination tests a few mosaic seedlings were obtained from clean as well as from mottled seeds.

Ordinarily the seed from plants apparently free from mosaic has yielded only healthy seedlings. In a field plot in 1922 planted with such seed, no mosaic occurred among the 590 seedlings, while in another

plot planted with seed from mosaic plants, 172 out of 993, or 17 per cent, of the seedlings were mosaic. Owing to the drought of 1922, the field symptoms were not easily recognizable at the time the seed was collected, and among 42 single plant selections from supposedly healthy plants, 3 showed mosaic when tested.

It would seem, therefore, that seed selection from mosaic-free plants may be fairly effective, but not absolutely infallible, as a control measure.

SECONDARY SPREAD OF MOSAIC

The degree of spread of mosaic during the season of 1921 is shown in Table I in which the number of mosaic plants on August 16 is very greatly in excess of those noted on June 26. In the Midwest variety this secondary infection involved 29 per cent of the stand. In another plot of the same variety, 37 per cent of the stand became infected during the month between July 19 and August 19.

The spread of infection in 1922 was not so extensive as in 1921. In field plots, 14 per cent of the 2,174 Midwest plants, 16 per cent of the 487 Haberlandt plants, and 1 per cent of the 640 Arlington plants became infected during the season.

During 1923 the spread of infection was much more extensive than in the two preceding seasons. Among variety plots equally exposed to infection and showing no mosaic among the seedlings, the extent of secondary spread of mosaic is evidenced by the percentages of mosaic recorded August 7 to 14, as shown in Table V. The varieties Soysota and Virginia seemed to escape infection.

TABLE V.—*Secondary spread of mosaic, 1923*

Variety.	Number of plants.	Per cent mosaic.	Variety.	Number of plants.	Per cent mosaic.
Midwest	181	90	Brown	220	41
Manchu	24	79	Lexington	92	40
Haberlandt	76	73	Pinpu	50	38
Elton	28	64	Black Eyebrow	30	33
Feldun	43	63	36847	30	33
Dunfield	38	55	Mammoth Yellow	79	23
Wea	60	45	Tar Heel Black	253	22
Arlington	61	44	Wilson Black	329	14
Ito San	211	41	Soysota	163	2
A. K	39	41	Virginia	308	0.6

The agent of dissemination has not been determined. Aphids have not been noted to any extent on the soybeans. Leafhoppers and tarnished plant bugs have been found in abundance, but numerous tests with caged plants have failed to incriminate either of these insects.

EFFECT OF MOSAIC ON SEED GERMINATION

Dickson (*3, p. 18–19*) has shown that there is a reduction in the germinating power of seed due to the mosaic disease in the case of red clover and Canada field pea, and Cunningham (*2, p. 27*) states that seed from mosaic bean plants has a low germinating quality. In the greenhouse tests with seed from mosaic soybean plants presented in Table II there was 79 per cent germination of the 676 Midwest seeds planted,

80 per cent germination of the 435 Haberlandt seeds, and 88 per cent germination of the 151 Feldun seeds. Although lower percentages of germination occurred in some of the other varieties, there was no marked reduction of germinating power in the larger lots of seed nor was the germinability in any way correlated with the percentage of mosaic in the seedlings. The latter also holds true for older seed as shown in Table IV.

In field plots planted in 1921 with 370 seeds from mosaic plants only 61 per cent germinated. The relative germinability of the seed from mosaic and healthy plants grown in 1922 was tested in the greenhouse and the results, as shown in Table VI, indicate that mosaic had little if any influence on the germinating power of seed.

TABLE VI.—*Effect of mosaic on the germinating power of seed*

Variety.	Seed from healthy plants.			Seed from mosaic plants.			
	Number of plants.	Number of seeds.	Per cent germination.	Number of plants.	Number of seeds.	Per cent germination.	Per cent mosaic.
Haberlandt	8	176	74	8	137	71	7
A. K	2	45	78	3	21	81	12
Lexington	3	80	66	3	39	59	0
Feldun	2	51	63	2	39	54	0

EFFECT OF MOSAIC ON YIELD OF SEED

Reddick and Stewart (7) found that bean mosaic suppressed seed production, and Dickson (*3, p. 18–19*) has recorded a marked reduction in yield of seed due to mosaic in pea beans, broad beans, and red clover. As the writers (*5*) have previously reported, reduction in yield of seed is likewise one of the outstanding features of soybean mosaic. In the fall of 1921 a comparison was made of the yield of seed from mosaic and normal plants and the results, as presented in Table VII, show that mosaic caused a very serious reduction in yield, especially in the Haberlandt variety.

The comparative yield of healthy and diseased plants in the variety plots in 1922 is shown in Table VIII. The mosaic seedlings had been tagged so that it was possible to record whether the disease was of seed origin or the result of secondary infection during the season, and except in the Midwest variety, the mosaic was all of the latter type.

TABLE VII.—*Effect of mosaic on yield of seed, 1921*

Variety.	Healthy.		Mosaic.		
	Number of plants.	Average yield per plant in grams	Number of plants.	Average yield per plant in grams.	Per cent reduction in yield.
Midwest	24	14.59	27	9.58	34
Haberlandt	5	41.57	9	10.96	76
Arlington	3	7.65	3	4.60	40
Lexington	2	12.01	2	4.82	60

TABLE VIII.—*Effect of mosaic on yield of seed, 1922*

Variety.	Condition of plants.	Number of plants.	Average number seeds per plant.	Average yield per plant.	Reduction in yield.	Average weight of a seed.
				Gms.	*Per cent.*	*Gms.*
Midwest	Healthy	28	18.0	1.85	.....	0.103
	Mosaic (seed origin)	95	4.4	.46	75	.105
Haberlandt	Healthy	22	22.0	3.01	.....	.137
	Mosaic	13	14.7	2.04	32	.138
Feldun	Healthy	6	21.6	3.25	.....	.141
	Mosaic	5	14.0	1.77	45	.126
Lexington	Healthy	7	22.8	2.00	.....	.151
	Mosaic	6	12.5	.93	53	.075
Dunfield	Healthy	4	35.7	4.65	.....	.130
	Mosaic	4	13.2	1.47	68	.111
Arlington	Healthy	6	40.8	2.56	.....	.062
	Mosaic	4	6.7	.23	93	.033
Manchu	Healthy	3	19.3	2.40	.....	.124
	Mosaic	3	10.3	1.10	54	.106
A. K.	Healthy	9	27.2	3.75	.....	.137
	Mosaic	8	9.6	1.14	69	.118

Owing to the drought, all of the yields were very low in 1922 as compared with 1921. However, the results in Table VIII show that mosaic caused heavy losses in all of the varieties. The loss in the Midwest variety, of which the greatest number of mosaic plants were available, amounted to 75 per cent. All of these Midwest plants represented mosaic of seed origin and 37 of the 95 bore no seeds whatever. In the Haberlandt variety, however, the average yield of seven plants with mosaic of seed origin, not recorded in Table VIII, was only 14 per cent less than that of the healthy plants.

It will be noted that in general the loss was due to the fewer number of seeds per plant rather than to the smaller size of the seeds, although in the last six varieties the seeds from mosaic plants were considerably smaller, especially in the Lexington and Arlington varieties.

SUMMARY

No host for soybean mosaic has been found other than the soybean itself.

Varieties of soybean seem to differ somewhat in susceptibility. Midwest has proved very susceptible; Soysota and Virginia have shown a tendency to escape infection.

Generally about 10 to 25 per cent of the seed from mosaic plants produced mosaic seedlings.

Varieties seem to differ in their ability to transmit mosaic through the seed. Midwest, Haberlandt, Black Eyebrow, A. K., and Arlington readily transmitted the disease.

Marked differences occurred in the percentages of mosaic in the progenies of individual mosaic plants of the same variety.

The transmission of mosaic seems to bear little or no relation to the location of the node at which the seed was produced nor to the date of infection of the parent plant.

The disease has been found in 2-year-old seed saved from mosaic plants.

A conspicuous brown mottling of the seed coat has not been correlated with mosiac.

Seed selected from mosaic-free plants gave rise to mosaic-free seedlings.

A considerable spread of mosaic occurred during the growing season. This secondary spread was more extensive in some seasons than in others.

Apparently the disease did not materially lower the percentage of seed germination.

Mosaic reduced the yield of seed 30 to 75 per cent. The number of seeds per plant was greatly reduced; in fact, plants with mosaic of seed origin frequently bore no seeds whatever.

LITERATURE CITED

(1) ARCHIBALD, E. S.
1921. REPORT OF THE ACTING DOMINION BOTANIST. *In* Rpt. Dominion Exp. Farms (Canada), 1919–20, p. 58-64.

(2) CUNNINGHAM, G. C.
1922. REPORT OF DOMINION FIELD LABORATORY OF PLANT PATHOLOGY, FREDERICTON, N. B. *In* Interim Rpt. Dominion Botanist, Canada Exp. Farms, 1921–22, p. 23-30.

(3) DICKSON, B. T.
1922. STUDIES CONCERNING MOSAIC DISEASES. MacDonald College (Quebec) Tech. Bul. 2, 125 p., 8 pl.

(4) DOOLITTLE, S. P., AND GILBERT, W. W.
1919. SEED TRANSMISSION OF CUCURBIT MOSAIC BY THE WILD CUCUMBER. *In* Phytopathology, v. 9, p. 326–327.

(5) GARDNER, Max W., and KENDRICK, James B.
1921. SOYBEAN MOSAIC. *In* Jour. Agr. Research, v. 22, p. 111–114, pl. 18–19.

(6) NEWHALL, Allan G.
1923. SEED TRANSMISSION OF LETTUCE MOSAIC. *In* Phytopathology, v. 13, p. 104–106. Literature cited, p. 106.

(7) REDDICK, Donald, and STEWART, V. B.
1919. ADDITIONAL VARIETIES OF BEANS SUSCEPTIBLE TO MOSAIC. *In* Phytopathology, v. 9, p. 149–152.

(8) ——— ———.
1919. TRANSMISSION OF THE VIRUS OF BEAN MOSAIC IN SEED AND OBSERVATIONS ON THERMAL DEATH-POINT OF SEED AND VIRUS. *In* Phytopathology, v. 9, p. 445–450.

(9) WIANCKO, A. T., and MULVEY, R. R.
1922. SOYBEANS IN INDIANA. Ind. Agr. Exp. Sta. Bul. 238 (revised), 16 p., 8 fig.

INSECTICIDAL EFFECT OF COLD STORAGE ON BEAN WEEVILS[1]

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INTRODUCTION

The present paper is a report on the insecticidal efficiency of certain commercial cold-storage temperatures upon our two most destructive insect pests of stored legumes, *Bruchus obtectus* Say, the common bean weevil, and *Bruchus quadrimaculatus* Fab., the four-spotted bean weevil. The effectiveness of cold in protecting beans, peas, and cowpeas against weevil attack has long been recognized, but relatively little attention has been given by investigators to the use of low temperatures for killing weevils which are present in stored legumes.

Duvel (*2*)[3] found that refrigeration at 32° to 34° F. was a perfect protection. Krall (*4*) concluded that eggs as well as all stages of the cowpea weevil, *Bruchus chinensis* L., can be killed by storage at 32° F. or colder (duration of exposure not given). Garman (*3*) stated that a temperature of 32° F. suspends the activity of the weevils and in time may kill them, although short exposures to this temperature apparently have no killing effect. He also concluded that all stages including eggs may be killed by one night's exposure to zero weather. The foregoing writers found that the germinating power of the seeds is not injured by refrigeration, even (*2*) for 20 months. Back and Duckett (*1*), Severin (*5*), and others stated that little or no development takes place at or below 50° F.; and Garman (*3*), speaking of temperatures of 35° to 40° F., wrote that "even these temperatures stop the work of the insects, though they will not entirely destroy them."

The present report summarizes experiments with *Bruchus obtectus* in California pink beans (*Phaseolus vulgaris*) and with *B. quadrimaculatus* in black-eye cowpeas or beans (*Vigna sinensis*). The results of work with the two species are here considered separately.

COLD-STORAGE EXPERIMENTS WITH BRUCHUS OBTECTUS

SCOPE AND METHOD

These experiments included the refrigeration of 92,484 California pink beans infested with an estimated total of 114,200 larvæ, pupæ, and adults. The temperatures tested, 32° and 36° F., were obtained in a modern cold-storage plant at Stockton, Calif., where inspection of the rooms by employees every two hours served to keep the desired temperatures fairly constant. The term "constant temperature" is used here in the commercial sense, and it is assumed that there were occasional

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[3] Reference is made by number (italic) to "Literature cited," p. 105.

fluctuations of a few degrees. Cotton sample bags were filled with from 3,000 to 5,000 infested beans each, obtained from commercial storage, and these were removed at intervals and mailed to the laboratory at Alhambra, Los Angeles County, Calif. The time spent in transit was sufficient for the insects not killed to become active.

A short time after they were received at the laboratory the beans were counted and a sample of 200 taken at random from each sack. These were dissected and all weevils found—larvæ, pupæ, and unemerged adults, both alive and dead—were recorded. Subsequent examinations of the sacks of beans were made at various times.

RESULTS WITH STORAGE AT 32° F.

Eleven samples of beans were exposed at 32° F. for periods ranging from 7 to 61 days. When the sacks were opened numerous emerged adult weevils were found. Among the 43,281 beans in the 11 samples, 5,649 adults were counted, 5,272 dead and 377 alive. No live adults occurred in samples refrigerated for 46, 56, or 61 days. Samples exposed for 7, 14, 22, 28, and 31 days contained sufficient live emerged weevils to reinfest the seeds. Although the condition of the emerged insects found is a rough index of the effect of the various exposures, it is necessary to refer to the results of subsequent examinations in order to ascertain the ability of the insects which survived within the beans to perpetuate the infestation.

At the time of the first examination, all emerged weevils were removed from the sacks. Reexaminations showed that some of the larvæ, pupæ, and adults which were alive within the beans at that time were able to emerge, following exposure to laboratory temperatures favorable to growth and reproduction. Heavy reproduction took place in beans refrigerated for 7 days, and live weevils were found in sacks subjected to 14 and 28 days of cold storage at 32° F.

The living weevils taken from a number of the samples at the first examination were placed under conditions favorable for reproduction, and it was found that adults from samples refrigerated 14 days or more were very weak and seemed to have lost much of their vitality; those from beans stored 22 days or longer failed to deposit eggs. Six other samples, not included in the tabulations given in this report, were exposed to 32° F. for 66 days and all emerged weevils were found to be dead. These samples were estimated to contain 20,500 beans and 43,700 weevils. A later inspection showed that this exposure had completely killed the infestation.

Table I gives the results of the dissection of 200 beans from each of the refrigerated sacks. A total of 2,200 beans dissected to represent the average condition of the 11 samples contained 2,360 weevils; 1,324 (56 per cent) of them were larvæ; 630 (27 per cent) were pupæ, and 406 (17 per cent) were adults which had not emerged from the pupal cells. The percentages of all forms killed after different periods of refrigeration are seen to be not very consistent with the increasing mortality to be expected with increasing exposure to cold. The samples were put in storage at two different times of year, November and February, and this seems to be related to the resistance of the insects, those subjected to refrigeration in November being less resistant than the others. The samples in which the mortality was 69.54, 71.48, and 97.20 per cent were

taken in November from a warehouse where the temperature was about 65° F., while those showing mortalities of 40.43, 52.17, 68.86, 80.45, and 85.13 per cent went into cold storage from a lower temperature in February.

In Table I the perfect control obtained after 56 days at 32° F. is shown. Forty-six days' exposure did not kill all the weevils within the beans, although, as previously stated, no live emerged adults were found.

TABLE I.—*Effect of a constant temperature of 32° F. on Bruchus obtectus*

Number of days in storage.	Larvæ.			Pupæ.			Unemerged adults.			Mortality, all forms.
	Number alive.	Number dead.	Mortality.	Number alive.	Number dead.	Mortality.	Number alive.	Number dead.	Mortality.	
			Per cent.			*Per cent.*			*Per cent.*	*Per cent.*
7	56	154	73.73	25	55	68.75	25	33	56.90	69.54
14	15	91	85.85	30	52	63.41	38	65	63.11	71.48
14	14	6	30.00	3	6	66.66	11	7	38.89	40.43
22	16	29	64.44	11	4	26.66	6	3	33.33	52.17
28	1	284	99.65	1	152	99.35	12	51	80.95	97.20
31	21	32	60.38	6	22	78.57	6	19	76.00	68.86
37	14	64	82.05	11	34	75.56	1	9	90.00	80.45
46	5	36	87.80	3	16	84.21	3	11	78.57	85.13
56	0	185	100.00	0	82	100.00	0	46	100.00	100.00
56	0	265	100.00	0	91	100.00	0	29	100.00	100.00
61	0	36	100.00	0	26	100.00	0	31	100.00	100.00
Total...	142	1,182	.....	90	540	.....	102	304	.....	.....

RESULTS WITH STORAGE AT 36° F.

Seven samples of beans were refrigerated at 36° F. for periods ranging from 14 to 66 days. At the first examination of the samples, which contained 28,703 beans, 2,199 weevils were found emerged—2,092 were dead and 107 alive. Samples exposed for 14, 22, 31, 37, and 46 days contained a sufficient number of live adults to reinfest the seeds. No live adults occurred among beans stored for 61 and 66 days.

Subsequent examination of these samples showed that new emergence had taken place from beans stored for 14, 22, 31, and 37 days, and that none had occurred in the 46 and 61 day samples; but one live adult was found to have emerged from beans stored 66 days at 36° F. As shown in Table II, 200 beans from this sample contained no live larvæ, pupæ, or adults, and the presence of one live adult in the sack 3 months after removal from cold storage is regarded as accidental.

The live weevils removed from samples at the time of the first examination were kept under favorable conditions to test their powers of reproduction. A few eggs were laid by females refrigerated for 32 days or longer, but all of these failed to develop. This fact tends to minimize the importance of the presence of a few feeble survivors.

Table II gives the results of dissecting 200 beans from each sample stored at 36° F., and shows (as with 32° F.) that the length of exposure which killed all emerged adults was not sufficient to give perfect control of forms within the beans. As previously stated, 61 days' refrigeration killed all emerged weevils, although some survived within the beans. The

1,400 beans dissected contained 792 weevils, and the larvæ showed a slightly greater percentage of mortality than pupæ and a considerably greater percentage of mortality than unemerged adults. Judging from the conditions found in the dissected beans, 66 days at 36° F. killed all weevils.

TABLE II.—*Effect of a constant temperature of 36° F. on Bruchus obtectus*

Number of days in storage.	Larvæ.			Pupæ.			Unemerged adults.			Mortality, all forms.
	Number alive.	Number dead.	Mortality.	Number alive.	Number dead.	Mortality.	Number alive.	Number dead.	Mortality.	
			Per cent.			*Per cent.*			*Per cent.*	*Per cent.*
14	4	22	84. 61	11	21	65. 62	7	17	70. 83	73. 17
22	25	28	52. 83	4	18	81. 82	7	12	63. 58	61. 70
31	11	17	60. 71	3	27	90. 00	9	19	67. 86	73. 25
37	6	21	77. 78	8	13	61. 90	10	13	56. 52	66. 20
46	7	24	77. 42	2	18	90. 00	7	17	70. 83	78. 67
61	0	20	100. 00	1	25	96. 15	5	23	82. 14	91. 89
66	0	220	100. 00	0	58	100. 00	0	32	100. 00	100. 00
Total...	53	352	.....	29	180	.....	45	133	.....	.....

COLD STORAGE EXPERIMENTS WITH BRUCHUS QUADRIMACULATUS

SCOPE AND METHOD

The resistance to cold of the eggs of the four-spotted bean weevil was tested by refrigerating 40,000 eggs (estimated) on about 6,000 black-eye cowpeas, with controls estimated at 13,300 eggs on 2,000 black-eye cowpeas. The eggs were deposited during a period of a few days before the seeds were placed in refrigeration, and had not begun to hatch at that time. The cowpeas on which the eggs had been deposited were placed in four thin cotton sample bags, each containing about 4,000 seeds.

The effect of cold upon the other stages of this insect was investigated by the cold storage of 12,000 black-eye cowpeas containing an estimated total of 36,000 weevils, with a control of 4,000 seeds infested to the same degree. Four sample bags were filled with the weevily cowpeas, each bag containing about 4,000.

One bag of cowpeas bearing eggs and one containing weevily cowpeas were left in the laboratory as controls, and similar pairs of bags were placed in each of the temperatures tested: 20°, 32°, and 39° F. The refrigerated rooms were in a modern cold-storage plant at Pasadena, Calif., where the temperatures were not permitted to vary more than 1° F. either way.

The weevily cowpeas used in the trials were taken from a 100-pound bag which was "heating" because of heavy infestation; the temperature within the bag varied from 95° to 100° F. (14 to 35° F. above room temperatures when the readings were made). About 30 minutes elapsed between the time the samples were taken from the laboratory and their storage in the cold rooms.

The cowpeas were examined at intervals, at which times samples of 50 or of 100 seeds were taken from each bag and transferred to the laboratory, where they were kept at temperatures favorable to growth and reproduction.

EFFECT OF 32 DAYS' REFRIGERATION ON SUBSEQUENT EMERGENCE OF ADULTS

The emergence of adults from three of these small lots after 32 days' exposure to 20°, 32°, and 39° F. is shown in Table III.

TABLE III.—*Emergence of Bruchus quadrimaculatus from black-eye cowpeas refrigerated 32 days*

Temperature of refrigeration.	Interval between removal from cold storage and examination.	Number of weevils emerged.			Temperature of refrigeration.	Interval between removal from cold storage and examination.	Number of weevils emerged.		
		Male.	Female.	Total.			Male.	Female.	Total.
°F.	Days.				°F.	Days.			
20.....	7	0	0	0	39.....	7	2	1	3
20.....	16	0	0	0	39.....	16	0	0	0
20.....	23	0	0	0	39.....	23	2	2	4
20.....	31	0	0	0	39.....	31	0	0	0
20.....	52	0	0	0	39.....	52	0	0	0
20.....	75	0	0	0	39.....	75	0	0	0
32.....	7	0	0	0	Control .	7	7	6	13
32.....	16	0	0	0	Do.	16	10	4	14
32.....	23	0	0	0	Do.	23	7	8	15
32.....	31	0	0	0	Do.	31	25	14	39
32.....	52	0	0	0	Do.	52	21	19	40
32.....	75	0	0	0	Do.	75	29	21	50

The data in Table III indicate that 32 days of refrigeration at 20° or 32° F. will kill all forms, but that a longer time at 39° is necessary to kill all infestation within the cowpeas.

EFFECT OF LOW TEMPERATURES ON IMMATURE STAGES OF WEEVILS

These low temperatures cause a dark spot to appear in the middle of the dorsal region of the larvæ. As this spot enlarges it becomes surrounded by a lighter band, and when approximately the middle one-fourth of the dorsal region becomes discolored the tissues beneath the integument soften and the larva dies. The same signs of injury can be observed in young pupæ.

EFFECT OF REFRIGERATION ON LARVÆ, PUPÆ, AND UNEMERGED ADULTS

As previously noted, a small lot of cowpeas was removed from each bag in refrigeration at the time of each examination. Some of these were dissected a few days after refrigeration; others were kept as long as seven months. Table IV gives the data obtained by the dissection of these samples. The dissection of 13 cowpeas four days after removing them from refrigeration at 20° F. for 11 days showed 20 per cent of the weevils to be alive, but the other 37 cowpeas of the same sample subsequently produced no adults. This indicates that although 20° F. does not kill all the unemerged weevils immediately, it injures them to such an extent that they die before emerging.

The indications of the foregoing data are that weevils emerge from cowpeas refrigerated at 39° F. for 11 and 18 days, respectively, but not after exposure to this temperature for 25 days (though this is

not in agreement with the results of 32 days' exposure to 39° F., as shown in Table III). Eleven days' exposure to 32° or to 20° F. gave a complete kill, judging from the conditions found in the samples, while 18 days' exposure to 39° F. did not kill the infestation.

TABLE IV.—*Results of dissection of refrigerated cowpeas*

Temperature.	Duration of refrigeration.	Interval between removal from refrigeration and dissection.	Number of cowpeas dissected.	Stages dissected from cowpeas.								
				Adults.			Pupæ.		Larvæ.		Total.	Dead.
				Alive.	Dead.	Emerged before dissection.	Alive.	Dead.	Alive.	Dead.		
°F.	*Days.*	*Days.*										*Per cent.*
20	4	10	50	3	7	0	7	8	9	27	61	69
20	11	4	13	0	6	0	2	5	1	1	15	80
		Months.										
20	11	7	37	0	8	0	0	10	0	100	118	100
20	18	7	50	0	17	0	0	33	0	106	156	100
20	25	7	50	0	20	0	0	40	0	89	149	100
20	32	6	100	0	34	0	0	82	0	184	300	100
32	11	6	50	0	15	0	0	29	0	87	131	100
32	18	6	50	0	19	0	0	52	0	94	165	100
32	25	6	50	0	23	0	0	65	0	72	160	100
32	32	6	100	0	28	0	0	83	0	236	347	100
39	11	6	50	0	12	8	0	37	0	97	154	95
39	18	6	50	0	22	4	0	42	0	88	156	97
39	25	6	50	0	21	0	0	47	0	80	148	100
39	32	6	100	0	18	0	0	70	0	261	349	100
Control		4	100	3	9	48	93	17	11	121	302	49

EFFECT OF REFRIGERATION ON THE VIABILITY OF THE EGGS

Table V shows that four days' refrigeration at 32° or 20° F. is sufficient to prevent all eggs from hatching; a temperature of 39° F. for the same length of time, however, leaves some eggs viable. Of the eggs removed from a temperature of 39° F. after four days' refrigeration and exposed to favorable temperatures, three hatched and one of the three larvæ developed and emerged.

TABLE V.—*Effect of refrigeration on the viability of eggs of Bruchus quadrimaculatus*

Duration of refrigeration.	Temperature.	Number of beans removed.	Number of eggs.	Number hatched.	Duration of refrigeration.	Temperature.	Number of beans removed.	Number of eggs.	Number hatched.
Days.	°F.				*Days.*	°F.			
4	20	50	[a]	0	25	32	50	[a]	0
11	20	50	[a]	0	32	32	100	656	0
18	20	50	[a]	0	4	39	50	[a]	[b] 3
25	20	50	[a]	0	11	39	50	[a]	0
32	20	100	664	0	18	39	50	[a]	0
4	32	50	[a]	0	25	39	50	[a]	0
11	32	50	[a]	0	32	39	100	697	0
18	32	50	[a]	0	Control		100	662	456

[a] Not counted. [b] Produced 1 adult.

A comparison of Tables IV and V shows that the eggs are more easily killed by low temperatures than the more advanced stages.

SUMMARY

BRUCHUS OBTECTUS

Exposure of larvæ, pupæ, and adults of *Bruchus obtectus*, infesting California pink beans, to 32° F. for 56 days or to 36° F. for 66 days gave very satisfactory control. Considerably shorter periods of exposure rendered the surviving adults incapable of reproduction.

BRUCHUS QUADRIMACULATUS

A temperature of 39° F. for 32 days was insufficient to kill all stages within black-eye cowpeas. All stages were killed when subjected to 32° F. or colder for 32 days.

The eggs were killed by four days' refrigeration at 32° or 20° F., but a longer time was necessary to kill them at 39°. They were more susceptible to cold than the other stages.

LITERATURE CITED

(1) BACK, E. A., and DUCKETT, A. B.
1918. BEAN AND PEA WEEVILS. U. S. Dept. Agr. Farmers' Bul. 983, 24 p., 24 fig.

(2) DUVEL, J. W. T.
1905. COLD STORAGE FOR COWPEAS. *In* U. S. Dept. Agr., Bur. Ent. Bul. 54, "Some miscellaneous results of the work of the Bureau of Entomology, VIII," p. 49–54, fig. 17, pl. 2–3.

(3) GARMAN, H.
1917. OBSERVATIONS AND EXPERIMENTS ON THE BEAN AND PEA WEEVILS IN KENTUCKY. Ky. Agr. Exp. Sta. Bul. 213, p. 305–333, 8 fig.

(4) KRALL, John A.
1914. A COWPEA WEEVIL (PACHYMERUS CHINENSIS). Okla. Agr. Exp. Sta. Circ. 31, 8 p.

(5) SEVERIN, H. C.
1919. THE BEAN WEEVIL. S. Dak. Off. State Ent. Circ. 15, 7 p., 2 fig.

THE EFFECT OF RUST INFECTION UPON THE WATER REQUIREMENT OF WHEAT[1]

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INTRODUCTION

During years of severe rust epidemics, such as those which the spring wheat sections of the northern Mississippi Valley and the prairie Provinces of Canada experienced in 1904 and 1916, and in other years, attempts have been made to estimate the loss suffered by the wheat crop as a direct result of the rust. Such attempts usually have been based upon the comparative yields in rust years and the average yield over a number of years in which rust was absent or not severe. It has not been possible to compare directly, on a large scale, the yields of rusted and rust-free fields where variety, soil type, and growing conditions are similar, because during rust epiphytotics nearly all fields are too generally affected. Furthermore, as a characteristic sequence of weather conditions usually occurs in rust years, it has been impossible wholly to differentiate the effect of rust from that of climate.

A scientific basis for apportioning the responsibility between the rust and the departures from normal weather is attainable only through the artificial maintenance of rusted and rust-free plots in which all circumstances are otherwise similar, and by noting the effect on reduction of yield as the several growth factors are varied. The factors which most require this sort of investigation are those associated with fertilization and moisture content of the soil, but an examination of the literature of the cereal rusts affords only meager information in respect to each.

As a beginning in this line of investigation, experiments were planned to determine the effect of rust infection upon the water requirement of the wheat plant and the influence of different conditions of nutrition on the water relations. It was believed that such data might be useful as a basis for separating the factors of climate and parasitism in the reduced yield of rusted wheat, particularly as regards the water requirement. It was desired further to compare the results of these cultures with those of soil-grown greenhouse and field plants in respect to the relation of fertilization to rust infection. Accordingly, this paper is divided into two parts, one of which deals with the effect of rust infection upon the water requirement, and the other with the influence of certain mineral nutrients on susceptibility to rust and injury from it.

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The investigation herein reported was conducted cooperatively between the Office of Cereal Investigations, U. S. D. A., and the Department of Agriculture of the University of Minnesota. It was undertaken as a part of the general project on the effect of soil nutrients on the development of stem rust and orange leaf rust of wheat.

EFFECT OF RUST ON WATER REQUIREMENT

REVIEW OF LITERATURE DEALING WITH EFFECT OF PARASITIC INFECTION ON THE WATER REQUIREMENT OF PLANTS

The literature of transpiration which relates to the effect of parasitic infection on the water use of the host is not extensive. Burgerstein (*6*)[1] cites the work of Müller-Thurgau (*11*) who, using the cobalt-chlorid paper method, measured the alteration in transpiration rate induced by infection of pear and apple leaves by scab and mildew fungi. Leaves infected by Fusicladium showed, in the diseased areas, increased transpiration as compared with adjacent healthy portions, especially if the upper leaf surface bore the lesion. Sphaerella, on the other hand, caused no marked change, while the lesions produced on grape leaves by *Peronospora viticola* (sic) lost almost no water at all, due to stoppage of the stomata by the conidiophores.

Blodgett (*2*) observed that a shoot of Rubus, heavily infected by Gymnoconia, took up nearly twice as much water as a comparable healthy branch, yet its leaves wilted while those of the latter remained turgid.

Reed and Cooley (*13*, *14*) measured the water loss from healthy apple leaves as compared with those infected by Gymnosporangium, making the determinations on leaves *in situ* and using an apparatus in which the transpired water could be absorbed and weighed. Under all conditions the amount of water transpired by infected leaves was less than that by healthy ones, the reduction being about 25 per cent. This was attributed to hypertrophy of the spongy parenchyma, reduction of intercellular spaces and the filling of these by fungus hyphae, and to the absence of stomata on the aecial cushions. At an early stage of infection the transpiration of affected leaves was not much less than that of sound ones. The reduction of transpiration was greatest at full development of the aecial cushions together with spore liberation. A rise in the rate of transpiration of rusted leaves occurred later when the lesions were surrounded by extensive areas of dead tissue. The possibility of an intoxicating effect on the host cells produced by fungous excretory products also was suggested.

Rust infection of cereals was found by Humphrey[2] and by Weaver (*22*) to cause accelerated transpiration. Weaver grew wheat, rye, barley, oats, and corn infected by stem and leaf rusts and compared the water loss under a variety of light and temperature conditions with that from uninfected controls. The amount of transpiration was determined by weighing the sealed pots at intervals and was expressed in grams per square centimeter of leaf surface. The rate was consistently higher in the rusted plants, the excess being greatest under environmental conditions favoring rapid water loss. A quantitative relation between acceleration of rate and area of the pustules was demonstrated. So slight an infection of oat leaves as involved only 0.5 per cent of the leaf area caused the transpiration rate to rise by 37 per cent. On the other hand dicotyledonous plants such as Xanthium, Helianthus, and Dianthus, when attacked by rust, either lost less water by transpiration than uninfected ones, or showed a slight acceleration when the pustules appeared, followed by a reduction if infection remained restricted to the original leaves.

[1] Reference is made by number (italic) to "Literature cited," pp. 117–118.
[2] HUMPHREY, H. B. Unpublished data cited by J. E. Weaver (*22*).

Reynolds (*15*) had previously described the effect of infection of apple leaves by Gymnosporangium, and of Xanthium by *Puccinia xanthii*, upon the tissue and cell morphology of the host. In general, the rusts were found to produce hypertrophic changes, but the separation from the mesophyll and the rupture of the epidermis at pustule formation exposed uncutinized cells to the drying effects of the atmosphere, resulting in atrophy. In Xanthium the loose organization of the mesophyll in healthy leaves is altered in rusted ones to a compact tissue composed of mycelium and empty host cells. The work of Ward, Evans, and others showed that in the grass rusts atrophic changes in the host cells do not appear until extensive development of the rust mycelium prior to pustule formation. In the case of resistant hosts, Stakman (*17*, *18*) found that disorganization of the chloroplasts followed by death and disintegration of the cells occurs in proximity to the invading germ tube.

Weaver rightly considered that rupture of the epidermis and death of the chlorenchyma cells in the infected area produce the marked acceleration in transpiration evident at pustule formation. He believed that toxic excretory products of the parasite also might stimulate transpiration, but are of slight importance in the cereal rusts. It was pointed out that transpiration may not always be an index of growth, for water loss from infected plants is excessive and bears no relation to the production of new substance. He did not determine the correlative transpiration, or amount of water transpired per unit of weight. The question of a sustained accelerating effect of rust infection on transpiration was raised, and the statement made that after the first pustules erupted no further change in rate was evident, the accelerated rate being maintained by the appearance of new pustules.

Dufrenoy (*8*) has given an interesting summary of the effects produced by a number of parasites and epiphytes on the transpiration of their hosts. Chlorotic areas of variegated leaves, and such as surround points of infection, transpire less rapidly than the green portions; the red-pigmented areas surrounding lesions caused by *Polystigma rubra* on plum leaves transpire more rapidly, contrary to the behavior of anthocyanin-containing cells generally. A number of parasites accelerate transpiration, owing to localized necrosis, rupture of the epidermis by fruiting bodies, or the production of overgrowths, although some witches' brooms appear to be xerophytic modifications. As the same effect sometimes is shown by epiphytes, changes in permeability of the host cells are involved also. A table is given which shows that among diverse hosts and parasites the transpiration of infected, as compared with healthy, leaves and parts of organs varies in a ratio of 0.5 to 1 to 100 to 1. The results were obtained by the use of hygroscopic paper.

It is to be noted that in none of the experimental demonstrations of acceleration of transpiration rate produced by parasitic infection which are reviewed above has the ultimate effect on the water economy of the host been determined. This would seem to be the most logical criterion of the general effect on the plant's water relations caused by parasitic attack.

Burkholder (*7*) has taken this into account in his study of the reduction in yield of beans resulting from infection by Fusarium rootrot. Infected plants gave only about half the dry weight of seed yielded by controls and transpired less water, the reduction amounting to about 50 per cent at soil temperatures of 18° and 26° C. Transpiration thus appeared to be an approximate index of growth.

Wimmer (*23*) showed that the water requirement of sugar beets and celery is increased by nematode infection, and believed this to be due to the slower growth of such plants. It is well known that the "compound interest law of plant growth" (*1*) falls far short of realization when growth is abnormally slow, and lowered water economy results also.

That parasitic fungi may affect the water relations of the host in other ways than by mechanical rupture of the tissues which normally protect the transpiring tissues, or by causing less economical use of water owing to retarded growth, is evident from the work of Haskell (*9*), who showed that in Fusarium wilt of potatoes, either the destruction of roots or the production of toxic excretory products by the fungus may be responsible for wilting of the plant, but mechanical stoppage of the vessels does not occur. The effect on water requirement is unknown.

Fungous infection may result either in acceleration or diminution of transpiration. The character and intensity of the effect are determined by the mechanical rupture of protective tissues, by local necrosis of the epidermis or mesophyll, or by toxic excretions, all of which result in accelerated water loss. On the other hand, transpiration may be diminished by hypertrophy of the chlorenchyma, reduction of air spaces, and number of stomata, by production of inhibitory excretions, by alteration of the osmotic relations of the infected cells and by injury to the water-absorbing parts.

The final or aggregate effect of rust infection on the water requirement of cereals has not been adequately determined. Since Weaver's results were secured with seedlings for a short growth period, it seemed that further work was necessary to obtain evidence in regard to the following three questions:

Is the transpiration of rusted wheat plants sustainedly greater than that of healthy plants throughout the growth period?

Is the amount of water transpired for the production of a unit weight of plant substance greater in rusted than in healthy plants, and are grain and straw production affected in the same way?

Is the effect on water requirement different in the case of infection by leaf rust and by stem rust, and is this effect related to differences in character and sizes of pustules?

EXPERIMENTAL METHODS

Marquis wheat was selected for the experiment, together with several strains of stem rust which produce large confluent pustules and no chlorosis on this host, and a mixture of collections of leaf rust to which this variety was readily susceptible. The plants were grown in Wausau quartz sand of a grade having a moisture equivalent of 3 per cent and a maximum water capacity of about 12 per cent. Crocks of one-half gallon capacity, provided with a drain at the bottom, served as pots. Two thousand eight hundred gm. of air-dry soil were placed in each. Three hundred cc. of nutrient solution were added, thus bringing the water content close to the maximum capacity, and one plant was set in each crock. The soil moisture content was maintained by weighing the crocks first at weekly and then at three-day intervals, and water was added to restore the base weight. The crocks were sealed with wax so that water loss was restricted to the plant. Although in the latter part of the growth period transpiration became very great, owing to the high diurnal temperatures in the greenhouse, there was no indication of

wilting at any time; and since the soil water-content was brought back to maximum at three-day intervals, it is believed that these plants made their growth under very favorable conditions of soil moisture. Therefore the reduced yield shown by infected plants should be attributed to the rust and not to lack of sufficient moisture for normal growth at any stage of development.

Mineral nutrients were supplied in solution as stated. The composition of the different nutrient solutions used is shown in Table I. As a basic solution Shive's (*16*) R_3C_3 was used containing $Ca(NO_3)_2$, KH_2PO_4 and $MgSO_4$ in the gram-molecular proportions 0.0078, 0.0108, and 0.0030,[4] respectively, and 5 cc. of ferric phosphate solution [Tottingham (*19*)] was added to each liter. Stock solutions of the salts were made up in half or quarter molecular concentration, and diluted at the time of using. The complete nutrient solution was made up fresh for each renewal.

To the basic nutrient solution, nitrogen as NO_3, phosphorus as PO_4, and potassium as chlorid, were added in two concentrations to form six modified solutions, the added salt in each case being one which did not introduce any cation under test. (See Table I.) The concentrations of added ions were of the order that would result from the application of 300 pounds of sodium nitrate, 420 pounds of sodium biphosphate, and 260 pounds of potassium chlorid per acre, for the lower concentration, and double these quantities for the greater concentration, assuming that all the added salts would be retained in the surface foot.

TABLE I.—*Composition in grams and gram-molecules per liter of the nutrient solutions used in 11 different cultures*

Component salts.	OO	OA	OB	IA	IB	IIA	IIB	IIIA	IIIB	IVB	VB
$Ca(NO_3)_2$:											
Gm.	1.279	1.279	1.279	1.279	1.279	1.279	1.279	1.279	1.279	1.279	1.279
Gm. mols.	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078
$NaNO_3$:											
Gm.				0.723	1.454						
Gm. mols.				0.0085	0.0171						
KH_2PO_4:											
Gm.	1.471	1.471	1.471	1.471	1.471	1.471	1.471	1.471	1.471	1.471	1.471
Gm. mols.	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108
NaH_2PO_4:											
Gm.						1.020	2.050				
Gm. mols.						0.0085	0.0171				
$MgSO_4$:											
Gm.	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361
Gm. mols.	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030
$MgCl_2$:											
Gm.											1.629
Gm. mols.											0.0171
$CaCl_2$:											
Gm.										1.896	
Gm. mols.										0.0171	
KCl:											
Gm.								0.634	1.274		
Gm. mols.								0.0085	0.0171		
NaCl:											
Gm.		0.500	1.000								
Gm. mols.		0.0085	0.0171								

A further modification of the basic solution was prepared by adding NaCl in the same molecular proportions as in the case of those salts of direct nutrient value in order to produce an osmotically equivalent [5]

[4] Shive's solution actually contained 0.0020 gm. mols. of $MgSO_4$, but the other of his two best solutions for wheat contained 0.0150; hence this slight change was made.

[5] Since these solutions were all prepared in gram-molecular rather than equivalent-molecular proportions, and since dissociation varies in the different salts, they are only approximately isosmotic. This is not considered to be significant as the solutions were employed in this experiment.

modification of the basic solution which contained no added ions of direct value. Two further modifications were prepared, one containing 0.0171 gram-molecules of $CaCl_2$ per liter, the other an equivalent quantity of $MgCl_2$. Eleven different solutions were used in all, each applied to four crocks.

The nutrient solution was renewed at intervals of three weeks by opening the drain at the bottom of the crock and flushing the sand with fresh solution. When the solution ceased to drain from the openings the crocks were weighed for a new base weight. As in all cases the amount of solution retained approximated the maximum water capacity of the soil, the successive base weights were in fairly close agreement.

The plants were grown from November to June. The long growth period and slow development during the winter resulted in a high water requirement as compared with field-grown plants. It was March before heading began and the end of May before the majority of the plants were ripe. It appears that length of day is an important factor in conditioning the time of maturity of greenhouse-grown wheat. It was necessary to cut the plants before all of them, particularly those in the excess N and K series, were fully ripe: the data from these are not considered in the summary, but a majority of the plants had plump hard grain at this time. The yield was rather below that of field-grown plants, the average for the three series of stem rust, leaf rust, and control plants translated into terms of bushels per acre being 9.6, 9.1, and 11.5, respectively. Certain cultures (four crocks), however, yielded at the rate of 20 to 25 bushels per acre and the best individual plant on a similar basis yielded 34 bushels per acre.

Three series of crocks were treated with each of the 11 culture solutions. One of these was left uninoculated as a control; in the second and third infection by stem rust and by leaf rust, respectively, was produced by artificial inoculation. Leaf-rust infection was readily obtained by atomizing the plants with a water suspension of urediniospores, but this method was unsuccessful with stem rust. With the latter, initial infections were produced by moistening the leaves and then applying dry urediniospores, following which steam was allowed to escape into the house for 48 hours while the ventilator remained open. In this way a nearly saturated atmosphere and dense fog could be maintained for two days at a time, resulting in copious precipitation of dew on the foliage. This process was repeated at monthly intervals to simulate the succession of new infections that occurs in the field. By these means a severe epiphytotic of stem rust was maintained, and a lighter one of leaf rust, during more than half of the growth period. In each case fairly uniform infection was produced on the replicate crocks of a culture, although some diversity in this respect could not be avoided.

The quantity of rust was estimated at the time of heading according to the rust scale of the Office of Cereal Investigations; but it is to be noted that at this time the amount of leaf rust was less than it had been at an earlier stage, while the stem rust was at a maximum. At maturity the tops were cut at the soil level, dried for two days at 105° C., and weighed. The grain was then hand threshed, dried again, and weighed. The data for the various cultures in each series are presented in Tables II and III. A direct comparison is thus permitted of a set of plants free from rust with a set each of leaf-rusted and stem-rusted plants which grew under identical conditions. Since there were only four plants in

each culture, great reliance can not be placed on these results; yet, taking into account the extent of agreement within each culture indicated by the computation of the probable error, certain significant differences appear to result from some of the treatments.

TABLE II.—*Yield and water requirement of Marquis wheat plants grown in each of ten cultures, consisting of four replicate crocks, in rusted and rust-free series*

Culture.	Yield.		Water used.	Water requirement.		Per cent rust.	
	Tops.	Grain.		Tops.	Grain.		
OO:	*Gm.*	*Gm.*	*Cc.*				
Stem rust	19.484±0.893	2.304±0.230	6,524±307	336± 5.0	3,079± 389	[a]25± 6.9	
Leaf rust	20.751± .812	2.638± .527	6,823±308	328± 5.9	2,676± 254	.....	[b]8±1.7
Control	19.219± .763	3.320± .220	6,625±208	346± 6.8	2,044± 131	.....	Tr.
OA:							
Stem rust	16.817±1.4	1.410± .238	6,226±247	380±20.0	7,645± 275	35±13	4
Leaf rust	18.884± .067	1.797± .280	6,451±244	342± 3.5	4,300± 192	.....	8± .94
Control	17.063±1.82	1.911± .560	6,348±163	327± 8.6	3,518± 53	.....	
OB:							
Stem rust	18.123± .36	1.717± .283	6,865±169	379± 1.7	5,630±1,646	40±11	
Leaf rust	21.550±1.26	1.862± .345	6,167±163	293±17.5	4,735±1,019	.....	9±1.3
Control	20.972± .491	2.453± .246	6,706± 43	324± 5.1	2,964± 369	Tr.	Tr.
IA:							
Stem rust	21.235± .378	1.670± .337	7,552±159	357±12.0	6,146±2,713	65± 5.4	
Leaf rust	24.496± .307	2.355± .503	7,145±219	291± 7.2	3,094± 154	.....	18±1.3
Control	23.711± .797	2.868± .323	6,625±129	259±13.0	2,948± 266	.....	Tr.
IB:							
Stem rust	21.138± .122	1.263± .339	6,785± 71	327±15.0	9,749±2,120	30± 4.9	
Leaf rust	23.953± .423	1.744± .288	6,633±161	277± 3.9	4,971±1,178	Tr.	32±1.6
Control	25.824± .433	1.123± .258	7,022±164	272± 2.9	8,148±1,275	.....	Tr.
IIA:							
Stem rust	19.563±1.55	1.931± .215	6,358±294	333±16.0	4,357± 987	25± 1.6	
Control	23.430± .507	1.375± .242	6,919±117	309± 3.5	6,252±2,169	Tr.	Tr.
IIB:							
Stem rust	16.924± .31	0.893± .46	6,109± 8	362± 7.6	7,973± 180	45± 6.8	
Control	19.677± .332	1.256± .396	6,443± 20.4	328± 9.1	5,518± 624	.....	1
IIIA:							
Stem rust	19.307± .824	1.598± .534	6,189±291	320± 5.3	4,159± 410	3± .67	
Leaf rust	18.379± .888	1.394± .266	5,983± 88	327± 6.8	5,598± 976	Tr.	2± .66
Control	21.635± .671	1.738± .392	6,646±150	307± 2.0	4,259± 874	.....	Tr.
IV:							
Stem rust	20.122± .535	3.356± .315	6,016±215	299± .18	1,937± 257	15± 2.9	
Leaf rust	19.121± .526	4.069± .390	5,445±242	285±10.0	1,406± 111	.....	[b]4± .67
Control	23.828± .373	5.605± .212	6,808±116	286± 4.2	1,223± 31	Tr.	1
VB:							
Stem rust	20.562±1.72	2.236± .442	5,816±111	293± 5.7	2,655± 756	8± 1.3	6± .94
Leaf rust	14.288±1.32	0.986± .438	4,588±337	327±14.0	5,293± 795	.....	1
Control	22.155± .573	1.885± .402	6,657±178	301± 4.5	3,619± 210	.....	

[a] Indicates stem-rust infection.
[b] Indicates leaf-rust infection.

TABLE III.—*Influence of stem rust and leaf rust on yield and water requirement of Marquis wheat, based on average results from ten different nutrient cultures, each consisting of four crocks* [a]

Culture.	Dry weight, mean.	Water used.	Water requirement.	Per cent rust.
Stem rust:	*Gm.*		*Cc. per gm.*	
Tops	19.327±0.345	6,444±109	339± 6.5	30±5
Grain	1.838± .139	.....	5,333±591	.....
Control:				
Tops	21.652± .526	6,679± 41	306± 5.8	.5± .2
Grain	2.353± .272	.....	4,004±459	.....
Leaf rust:				
Tops	20.178± .801	6,154±106	308± 8.7	10±1
Grain	2.015± .219	.....	4,009±386	.....

[a] Data from the immature plants, including culture IIIB in all series, and the plants in culture IIA and IIB of the leaf rust series are omitted.

DISCUSSION

An examination of Table III shows less difference in yield of tops and grain, and of water requirement based respectively on these, than might be expected. It is to be borne in mind, however, that the reduction in yield is not in this case complicated by diminution of the soil moisture to a dangerous point, as frequently occurs in the field when drought and rust are coincident. Furthermore, the prolonged growth period undoubtedly resulted in an abnormally high water requirement for all cultures, whether rust-infected or not, and tended to eliminate more marked differences that might have been evident in a shorter period of rapid growth.

In connection with their extensive researches on the water requirement of crop plants, Briggs and Shantz (*3, 4, 5*) summarized the results reported by earlier investigators. Thus the water requirement for wheat (tops), as reported in literature, ranged from 235 found by Lawes at Rothamstead to 554 found by Leather in India. The results here reported lie well within this range. Briggs and Shantz found the average water requirement for six varieties of wheat to vary from 400 to 500 in different years. The water requirement based on grain production, in the present instance, is abnormally high in nearly every case, being comparable to that reported originally by Briggs and Shantz for plants growing under conditions unfavorable to economical use of water, and more than twice the figures given in their later papers (*loc. cit.*). However, at least one culture, IVB of the control series, compares favorably with their results in all respects. The following conclusions seem to be warranted by the data:

Infection by either stem rust or leaf rust results in a significant reduction of yield of tops and of grain that is independent of the soil moisture supply, and this reduction is evident with only moderately heavy infection. Taking the figures for the stem rust and control series at face value, it appears that an amount of rust estimated at 30 per cent may cause a 20 per cent reduction in yield even though the soil moisture content remains at a favorable level.

Accompanying the reduction in yield, there is practically as great use of water as in healthy plants; that is, rusted plants have a higher water requirement, based on yield of both tops and grain. This is shown to be significant only in the case of stem rust and is of a higher order for grain than for total dry matter. The lesser effect on yield of tops, resulting from rust infection, is probably due to the fact that the soil water supply was at all times sufficient; under field conditions dry weather may greatly diminish the yield of the entire plant.

The effect of rust in diminishing yield and increasing the water requirement seems less likely to be due to increased transpiration, resulting from rupture of the epidermis at postule formation than to other causes, for the total amount of water used by rusted and healthy plants is about the same. Leaf rust, which produces but slightly erumpent pustules as compared with stem rust, and hence less injury to the epidermis, also depresses yield, more particularly that of grain, but also of tops, though in each case to a less extent. Chief among the causes of yield reduction arising from rust infection are to be reckoned the destruction of chloroplasts in the infected tissue and the drain on the elaborated food reserves of the host.

EFFECT OF CERTAIN MINERAL NUTRIENTS ON DEVELOPMENT OF RUST AND INJURY TO HOST

The original plan of this investigation included an extensive study of the effect of mineral nutrition upon the host as regards its susceptibility to rust infection and the injury it suffers therefrom. Owing to the fact that it was not possible to repeat the experiment described in Part I of this paper, or to extend the investigation along different lines, it seems best to draw no conclusions in respect to these questions from the limited data at hand. Certain tendencies were noticeable, however, as regards the performance of the plants under some of the treatments, and the data from all cultures which attained approximate maturity are presented in Table IV, arranged to show the effect of the mineral nutrients on the yield and quantity of rust.

TABLE IV.—*Influence of different culture media on average yield and water requirement of two cultures of Marquis wheat, one rusted and one rust-free, each consisting of four replicate crocks*

Culture.	Yield.		Water used.	Water requirement.		Average per centage of rust for two infected cultures.
	Tops.	Grain.		Tops.	Grain.	
	Gm.	*Gm.*	*Cc.*			
OO.....	19.818±0.822	2.754±0.326	6,657±274	336± 5.9	2,600± 258	17
OA.....	17.588±1.095	1.706± .361	6,342±218	350±107.0	5,154± 173	24
OB.....	20.215± .703	2.011± .291	6,579±125	332± 8.1	4,443±1,043	25
IA.....	23.147± .494	2.298± .387	7,107±169	302± 10.4	3,913±1,044	42
IB.....	23.638± .326	1.377± .261	6,813±132	292± 7.2	7,623±3,567	31
IIA[a].....	20.997±1.028	1.653± .228	6,639±205	321± 9.7	5,305±1,578	13
IIB[a].....	18.300± .321	1.075± .428	6,276±106	345± 8.3	6,746± 402	23
IIIA[b]...	19.612± .794	1.335± .397	6,267±176	320± 4.7	7,765± 753	3
IVB.....	21.024± .478	4.343± .305	6,090±191	290±104.0	1,522± 136	10
VB.....	19.002±1.204	1.702± .427	5,687±208	307± 8.0	3,856± 587	8

[a] Data for leaf rust series omitted owing to immaturity of the plants.
[b] Series IIIB, with excess KCl, omitted owing to immaturity.

Even a greatly curtailed review of the literature of transpiration dealing with the effects of nutrient ions and other substances and the state of fertilization upon the water relations of plants, will not be attempted here. Briggs and Shantz (*3*, *4*) have given a very thorough review of the literature dealing with water requirement based on mature growth of plants, and a bibliography of papers containing water requirement measurements based on seedlings. A large number of papers dealing only with rate of transpiration as affected by various substances, of nutritive value and otherwise, is included in the comprehensive review of the subject of transpiration by Burgerstein (*6*). The literature dealing with the effect of fertilization on the severity of rust is reviewed by Raines (*12*).

Briggs and Shantz conclude that a reduction in the water requirement results from the use of fertilizers, although very little difference in this respect is shown by highly productive soils. A highwater requirement may result from deficiency of a single nutrient element, or in fact from any condition which operates to limit or retard growth, as measured by carbon assimilation, while transpiration suffers no corresponding change. Furthermore, a highwater requirement may be

brought about by a soil moisture content which tends to be too low or too high for unrestricted growth, or, in the case of water cultures, by great dilution of the solution.

An examination of Tables II and IV shows, first as regards the yield of tops and grain obtained from the various nutrient treatments, that only cultures OO, OB, IA, and IVB have produced yields comparable to ordinary yields of field-grown plants—i. e., above 15 bushels per acre. Culture OO is similar to Shive's R_3C_3 which was found most suitable for growth of wheat seedlings during the first 30 days. It is to be noted that in yield of grain this culture was exceeded only by IVB containing $CaCl_2$. Even the addition of nitrate, an ion which Hoagland (*10*) has shown may be absorbed very rapidly from solutions and which may therefore be deficient, particularly during the period of greatest absorption, in sand or solution cultures carried on as these were, resulted only in increasing the yield of tops. The reason for the striking increase in grain produced by the addition of $CaCl_2$ to the Shive solution is not clear. It can hardly be attributed to the direct nutritive value of calcium, but is more likely to be accounted for either by an improvement in the reaction of the culture medium or by rendering other ions "physiologically available" (True, (*21*)). A depressing effect on plant growth resulting from too high acidity of the medium has been observed by a number of recent investigators, and it has been shown that a correlation exists between acidity of the cell sap and retarded growth (*20*). Whatever the explanation, it is significant in this case that the Shive solution, modified by the addition of 0.0171 gram molecules of $CaCl_2$ per liter, has produced a growth of mature wheat that in respect to yield and water requirement is comparable to that of plants grown under the best pot culture methods that have been devised or with that of field-grown wheat. The low-water requirements shown by these plants, both for tops and for grain, is in line with expectations if one of the functions of calcium, as stated by True (*21*), is to maintain normal permeability relations of the cell wall.

As regards the effect of mineral nutrition on the development of rust, it appears that the application of an excess of nitrate has resulted in greater susceptibility to rust in both the leaf-rust and stem-rust series. In Culture IA, where the additional nitrogen did not so greatly delay maturity, the infected plants show a much reduced yield of grain also. The appearance of the plants themselves, particularly in the leaf-rust set, confirmed their more ready susceptibility, as both the number and size of pustules were greater on plants receiving additional nitrogen. The more luxuriant development of culms and the more rapid growth of these plants seem sufficient to account for the greater development of rust, and until further evidence is at hand, the assumption of a physiologically greater susceptibility to rust in nitrogen-fertilized plants is unnecessary.

The slight development of rust on the plants receiving calcium and magnesium is noteworthy since in these cultures the effect can not be attributed to generally poor growth, which is the case in those fertilized with phosphate and potash. It appears that the plants of Cultures IV and V actually were somewhat physiologically resistant, since repeated attempts to infect them were only partially successful. Hurd[6] indicates

[6] HURD, Annie M., THE ACIDITY OF SOME RUST AND SMUT RESISTANT AND SUSCEPTIBLE VARIETIES OF WHEAT AND SOME FACTORS AFFECTING IT. Abstract presented at the meetings of the Physiological Section, American Bot. Soc., at Toronto, Canada, Dec. 29, 1921. (Mimeographed.)

a correlation between the total acidity of the cell sap of certain varieties of wheat and resistance to rust and smut; also that the total acidity (content of buffer substances) may be decreased by liming the soil. Should a similar correlation have existed in the plants constituting Culture IV of the present experiment, their "resistance" to rust must have been due to something other than the reaction of the sap.

SUMMARY

Marquis wheat was grown to maturity in quartz sand cultures supplied with various combinations of mineral nutrients added in solution. An artificial epiphytotic of leaf rust, *Puccinia triticina*, was induced in one series, and of stem rust, *P. graminis tritici*, in a second, while a third was maintained free from infection.

Rust infection of either type resulted in lowered water economy of the host, whether the dry matter of entire tops or of grain is considered. The actual quantity of water transpired is of significance in relation to infection only when the correlative production of dry matter is taken into account.

The addition of NaCl or NaH_2PO_4 to the basic three-salt nutrient solution did not affect the susceptibility of wheat to leaf rust or stem rust. The addition of $NaNO_3$ resulted in somewhat readier infection in each case but did not predispose to greater injury. KCl retarded infection in proportion to the diminution of growth of the host, particularly when used in excess. $CaCl_2$ and $MgCl_2$ appeared to induce a state in which the host was less readily susceptible to infection. $CaCl_2$ also resulted in a reduction of water requirement, about 10 per cent for tops and 40 per cent for grain.

The addition of 0.0085 and 0.0171 gram molecule of $NaNO_3$ per liter and 0.0171 gram molecule of $CaCl_2$ caused an increase in yield over that obtained from the Shive R_3C_2 solution as here used.

LITERATURE CITED

(1) BLACKMAN, V. H.
1919. THE COMPOUND INTEREST LAW AND PLANT GROWTH. *In* Ann Bot., v. 33, p. 353–360.

(2) BLODGETT, F. H.
1901. TRANSPIRATION OF RUST-INFESTED RUBUS. *In* Torreya, v. 1, p. 34–35.

(3) BRIGGS, Lyman J., and SHANTZ, H. L.
1913. THE WATER REQUIREMENT OF PLANTS. I. INVESTIGATIONS IN THE GREAT PLAINS IN 1910 AND 1911. U. S. Dept. Agr. Bur. Plant Indus. Bul. 284, 49 p., 2 fig., 11 pl. Literature cited, p. 49.

(4) ——— ———
1913. THE WATER REQUIREMENT OF PLANTS. II. A REVIEW OF THE LITERATURE. U. S. Dept. Agr. Bur. Plant Indus. Bul. 285, 96 p., 6 fig. Literature cited, p. 93–96.

(5) ——— ———
1914. RELATIVE WATER REQUIREMENT OF PLANTS. *In* Jour. Agr. Research, v. 3, p. 1–63, 1 fig., 7 pl. Literature cited, p. 62–63.

(6) BURGERSTEIN, Alfred.
1904–1920. DIE TRANSPIRATION DER PFLANZEN. 2. Teil. Jena. Literaturnachweise, Teil 1, p. 251–283; Teil 2, p. 236–264.

(7) BURKHOLDER, W. H.
1920. THE EFFECT OF TWO SOIL TEMPERATURES ON THE YIELD AND WATER RELATIONS OF HEALTHY AND DISEASED BEAN PLANTS. *In* Ecology, v. 1, p. 113–123, illus.

(8) DUFRÉNOY, J.
1918. LES FACTEURS PHYSIQUES DE LA TRANSPIRATION CHEZ LES PLANTES ET LA TRANSPIRATION DES FEUILLES PARASITÉES. *In* Rev. Gén. Sci., ann. 29, p. 565–566.

(9) HASKELL, R. J.
1919. FUSARIUM WILT OF POTATO IN THE HUDSON RIVER VALLEY, NEW YORK. *In* Phytopathology, v. 9, p. 223–260, pl. 13–15. Literature cited, p. 259–260.
(10) HOAGLAND, D. R.
1919. NOTE ON THE TECHNIQUE OF SOLUTION CULTURE EXPERIMENTS WITH PLANTS. *In* Science, n. s., v. 49, p. 306–362.
(11) MÜLLER-THURGAU, H.
1895. DIE TÄTIGKEIT PILZKRANKER BLÄTTER. *In* Jahresber. Vers. Stat. u. Schule Wädensweil, Bd. 4 (1893–94), p. 54–57.
(12) RAINES, M. A.
1922. VEGETATIVE VIGOR OF THE HOST AS A FACTOR INFLUENCING SUSCEPTIBILITY AND RESISTANCE TO CERTAIN RUST DISEASES OF THE HIGHER PLANTS. I. *In* Amer. Jour. Bot., v. 9, p. 183–203.
(13) REED, Howard S., and COOLEY, J. S.
1913. THE EFFECT OF GYMNOSPORANGIUM ON THE TRANSPIRATION OF APPLE LEAVES. *In* Va. Agr. Exp. Sta., Ann. Rpt. 1911–12, p. 82–90, fig. 16.
(14) ——— ———
1913. THE TRANSPIRATION OF APPLE LEAVES INFECTED WITH GYMNOSPORANGIUM. *In* Bot. Gaz., v. 55, p. 421–430, 1 fig.
(15) REYNOLDS, Ernest S.
1912. RELATION OF PARASITIC FUNGI TO THEIR HOST PLANTS. I. STUDIES OF PARASITIZED LEAF TISSUE. *In* Bot. Gaz., v. 53, p. 365–395, 9 fig. Literature cited, p. 390–395.
(16) SHIVE, John W.
1915. A STUDY OF PHYSIOLOGICAL BALANCE IN NUTRIENT MEDIA. *In* Physiol. Researches, v. 1, p. 327–397, 15 fig. Literature cited, p. 396–397.
(17) STAKMAN, E. C.
1914. A STUDY IN CEREAL RUSTS: PHYSIOLOGICAL RACES. Minn. Agr. Exp. Sta. Bul. 138, 9 pl. Bibliography, p. 50–54.
(18) ———
1915. RELATION BETWEEN PUCCINIA GRAMINIS AND PLANTS HIGHLY RESISTANT TO ITS ATTACK. *In* Jour. Agr. Research, v. 4, p. 193–200, pl. 28. Literature cited, p. 198–199.
(19) TOTTINGHAM, William E.
1914. A QUANTITATIVE CHEMICAL AND PHYSIOLOGICAL STUDY OF NUTRIENT SOLUTIONS FOR PLANT CULTURES. *In* Physiol. Researches, v. 1, p. 133–245, 15 fig. Literature cited, p. 242–245.
(20) TRUOG, Emil.
1918. SOIL ACIDITY: I. ITS RELATION TO THE GROWTH OF PLANTS. *In* Soil Sci., v. 5, p. 169–195.
(21) TRUE, Rodney H.
1922. THE SIGNIFICANCE OF CALCIUM FOR HIGHER GREEN PLANTS. *In* Science, v. 55, p. 1–6. Bibliographical footnotes.
(22) WEAVER, J. E.
1916. THE EFFECTS OF CERTAIN RUSTS UPON THE TRANSPIRATION OF THEIR HOSTS. *In* Minn. Bot. Studies, v. 4, p. 379–406, pl. 41–42. Literature cited, p. 406.
(23) WIMMER, G.
1908. NACH WELCHEN GESETZEN ERFOLGT DIE KALIAUFNAHME DER PFLANZEN AUS DEM BODEN? Arb. Deut. Landw. Gesell., Heft. 143, 169 p.

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